Beckman Model 150 Ultramicro Analytical System

INTRODUCTION

GENERAL

Need for Ultramicro Methods

In recent years, understanding has increased of the significance of the chemical constituents of the blood and other body fluids in the diagnosis and treatment of disease. Because many factors need to be known in order to assess a patient's condition accurately, larger volumes of blood are needed now than previously.

It is often undesirable, or even impossible, to remove large volumes of blood by venipuncture; as in babies, infants, the exceptionally obese, and diabetics. In order to do many tests on volumes of blood too small for the usual procedures, micro and ultramicro methods have been introduced. By means of the ultramicro methods, a whole series of tests can be done on an extremely small volume of capillary blood.

Development

The Beckman/Spinco Model 150 Ultramicro Analytical System has been developed from the techniques of a pioneer in ultramicroanalysis: Dr. M. C. Sanz of the Central Laboratory, University Hospital, Geneva, Switzerland.

Definition

Ultramicro methods are those in which the volume of material to be analyzed (*serum, urine, etc.*) is one to fifty microliters. However, the amount of component being measured can vary enormously.

SPECIAL CONSIDERATIONS IN ULTRAMICRO

It is fairly easy to change from macro to semimicro and micro work. More or less the same equipment is used, except that it is smaller. However, other considerations become important when volumes are reduced to the ultramicro scale. These include:

Surface Effects

The significant enlargement of surface relative to volume encountered in changing to work with very small volumes enormously increases all surface effects. The problems from this relative enlargement of surfaces are solved in the Spinco System by the use of non-wettable apparatus.

Denaturation of Proteins

Protein denaturation is greater on glass surfaces than on non-wettable surfaces and can cause significant errors when ultramicro quantities of proteins are analyzed in glass. Virtually, no denaturation of protein occurs at the liquid-air and liquid-nonwettable boundaries (surfaces) of the new system.

Immobilization and Concentration at Surfaces

Molecules are strongly bound at, or near, the surface of a solution and where liquid meets solid. The Spinco System's micro titration cups, micro test tubes, and the Microtitrator's burette delivery tip and stirring tip are all non-wettable. Vigorous stirring during titration takes care of surface binding.

Volume Losses by Evaporation

Evaporation from micro test tubes at room temperature is negligible for 30 minutes. Errors due to the more rapid evaporation during centrifugation are avoided by high-speed, short-duration centrifugation.

Cleanliness of Apparatus

For the little cleaning required in the Spinco System, Alconox or, rarely, hydrochloric acid is used, followed by a hot distilled water rinse. Surfaces which have been in contact with protein come clean when soaked in pepsin overnight. The ultramicro cuvette is kept scrupulously clean because its small volume could otherwise lead to large errors.

Airborne Impurities

Airborne impurities are to be avoided when working on the ultramicro scale; sodium, potassium, calcium, and ammonia being particularly troublesome. Ventilation should include effective filtration.

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Sparepak
The Sparepak (Spinco No. 158-900) contains spare Micropipettes of various volumes and additional Micro test tube racks.

The Micropipette
The Beckman/Spinco Micropipette semi-automatically delivers a constant volume of either sample or reagent.
Each Micropipette assembly consists of a plastic bottle and a dome, to which is attached a non-wettable, non-breakable polyethylene pipette. The pipette is self-adjusting and is filled and emptied by hand pressure against the sides of the bottle. The sample Micropipette is identical to a reagent Micropipette except that it needs no tube extending down into the bottle because its pipette is filled from an outside source. The volume delivered is the volume of the pipette fitted to the Micropipette assembly.
Accuracy of final results depends on reproducibility of all volumetric measurements. This reproducibility in turn depends on the precision with which menisci adjust themselves at ends of the pipettes.
The overflow pipettes in the Spinco System ensure precise location of menisci and are self-washing. Pipettes with the volumes required for all volumetric work of a procedure are supplied with each Micropak.

Spectro-Colorimeter
The Beckman/Spinco Model 151 Spectro-Colorimeter is a direct-reading combination spectrophotometer and colorimeter. It uses one stationary microcuvette for all readings. This cuvette is wettable to guarantee a continuous film which sucks off the cuvette wall more easily than single drops and avoids air bubbles. It is emptied by pressing a button to activate a suction pump. To guarantee wettability, the cuvette is cleaned after each series of readings by rinsing with detergent solution, distilled water, and, finally, a wetting agent. The Spectro-Colorimeter provides for wavelengths from 400 to 650 millimicrons. Its meter indicates both absorbance and per-cent transmittance.

Microfuge
The Beckman/Spinco Model 152 Microfuge is a high-force micro-centrifuge, with instant acceleration and deceleration. It spins down a small sample rapidly, reducing evaporation to a minimum. As many as 20 of the polyethylene micro test tubes can be centrifuged at one time. Tube holders and a tube rack are supplied with each Microfuge.

Microtitrator
The Beckman/Spinco Model 153 Microtitrator does routine titrations on small volumes. The burette, which contains the titration solution, has a micrometer-driven displacement piston which gives remarkable reproducibility. The volume of titration solution delivered is indicated on a large dial. This can be returned to zero after each titration by pressing the zero button.
A vigorous vibration stirrer is immersed in the solution during titration. There is no splashing of small drops. The positions of the delivery tip and stirring tip are adjustable.

Micromixer
The Beckman/Spinco Model 154 Micromixer thoroughly mixes liquids in the small containers. It is used, for example, to mix serum and reagents in a micro test tube.

Micro Test Tubes and Microtitration Cups
These are unbreakable and priced to be discarded after one use.

HOW TO SET UP AN ULTRAMICRO LABORATORY

A self-contained section of the general laboratory should be devoted exclusively to ultramicroanalysis. A relatively small space is required because of the small instruments. The components should be placed for convenience as experience dictates.

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ADVANTAGES OF THE SPINCO SYSTEM

1. Reproducibility and Accuracy

The Spinco System gives better reproducibility than the usual 5% of ordinary methods; in our laboratories we typically achieve within 1%. There are reasons for this. A single pipette is used for all blanks, standards and samples, and all deliveries of a particular reagent are made with the one pipette specific for that reagent.

The precision burette always delivers at a constant rate. A single, fixed optical cuvette eliminates physical variables in colorimetric measurements. Disposable test tubes and titration cups prevent errors due to dishwashing. Reagent variability is virtually no problem as hundreds of tests are done directly from one bottle of reagent.

2. Small Volumes

The Beckman/Spinco Model 150 Ultramicro Analytical System permits routine analysis of 5-, 10-, or 20-μl. samples. In many cases, the amount of sample required has been reduced from conventional practice by a factor of 40 or more. The Spinco System makes possible several different analyses on one small sample. For example, one-sixth of a cc. of serum is enough for these tests: glucose, urea nitrogen, chloride, calcium, cholesterol, indirect bilirubin, direct bilirubin, inorganic phosphate, and total protein.

3. Almost no Dishwashing

Practically all dishwashing is eliminated because pipettes are self-cleaning, and test tubes and other containers are thrown away because of low cost.

4. The System is Simple in Operation

5. Speed

Analysis is faster; semi-automatic pipetting eliminates most time-consuming volumetric measurements; centrifugation is rapid because of the small sample volume and instant acceleration and deceleration; colorimetric measurements are made in a single cuvette which is automatically flushed and cleaned between readings by touching a button.

6. Economy

The Spinco System is inexpensive both in initial cost and in operation. Valuable technician time is saved because of rapid operation. The System is economical of laboratory space: the area required for an analytical laboratory has been reduced from a room to a table top. The very much smaller volumes of reagents required significantly reduces chemical costs. The plastic ware does not break.

7. Ruggedness

The equipment is virtually unbreakable because all critical pieces are plastic.

8. Venipunctures are Avoided: Capillary Blood is Used

9. Integrated System

The Spinco System is completely integrated. Additional components and procedures will be offered as they are developed.

INSTRUMENTS AND OTHER COMPONENTS

The complete Model 150 Ultramicro Analytical System (Spinco No. 150-310) permits both colorimetric and titrimetric analyses. It consists of Spectro-Colorimeter, Microfuge, Microtitrator, Micromixer, Micropaks, Chempaks and Sparepak.

Instruments Now Available Are:

The Beckman/Spinco Model 151 Spectro-Colorimeter (*Spinco No. 151-100*)

The Beckman/Spinco Model 152 Microfuge (*Spinco No. 152-100*)

The Beckman/Spinco Model 153 Microtitrator *Spinco No. 153-100*)

The Beckman/Spinco Model 154 Micromixer (*Spinco No. 154-100*)

These operate on 115 volts, 50/60 cycles, but are available wired for other power requirements.

Other Components Now Available Are:

Micropaks

A Micropak is available for each of the colorimetric and titrimetric procedures. Each Micropak contains the equipment necessary for that procedure. This includes labeled Micropipettes of the correct volumes, a test rack, and a base and cover for holding the Micropipettes. Micropaks are now available for analyses for *calcium, chloride, bilirubin, cholesterol, creatinine, glucose, phosphorus, total protein, urea nitrogen, and uric acid.*

Chempaks

A user may provide his own chemicals or buy them from Spinco. The chemicals required for a particular procedure are contained in the appropriate Chempak.

Chempaks are now available for analyses for *calcium, chloride, bilirubin, cholesterol, creatinine, glucose, phosphorus, total protein, urea nitrogen, and uric acid.*

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Technical Bulletin No. UM-TB-003C

Beckman Model 150 Ultramicro Analytical System SPECTRO-COLORIMETER

INTRODUCTION

General Description

The Beckman Model 151 Spectro-Colorimeter is a direct-reading combination spectrophotometer and colorimeter. It is designed specifically to carry out routine clinical chemistry analyses; and is particularly well suited to the measurement of the absorbance of extremely small volumes of sample. The Model 151 Spectro-Colorimeter is speedy, simple to operate, and can be run continuously with negligible drift. Because of its straightforward design, it requires only minimum maintenance. Its ruggedness insures lasting stability and a long life of accurate measurements.

The Model 151 operates directly from a standard electrical outlet, and, since it is internally stabilized, does not require a voltage-stabilizing transformer accessory. Light is supplied by a low-wattage tungsten lamp of fixed focus. Wavelengths are available over the normal visual spectral range of 400 to 650 millimicrons: bandwidth is about 15 millimicrons. Selection of these narrow bandwidths of visible light is made by rotation of a knurled wheel which moves a glass-wedge interference filter. Alternatively, standard glass filters may be employed instead of the wedge interference filter.

The same stationary cuvette is used for all measurements: this insures that the depth through which the light beam passes remains constant and that the cuvette surfaces used for sample are identical to those used for standard. The photoelectric current is indicated by the meter in absorbance and in percent transmittance.

The instrument incorporates a vacuum pump which aspirates fluid out of the cuvette into a waste bottle: this permits samples to be introduced, measured and emptied with rapidity.

Three wash bottles contain liquids to clean the cuvette. The liquids in the wash bottles are, from left to right: (i) distilled water; (ii) 1% detergent solution; and (iii) 1% caprylic alcohol in acetone. Each bottle is labeled and has a dropper.

FUNCTIONS OF CONTROLS

(See Figure 1.)

Dark Current Knob

This control is the knob on the upper right-hand side of the control panel marked "Off/Zero."
It performs two functions:

(1) It turns the instrument on and off.
(2) It adjusts the needle on the meter to zero percent transmittance when the shutter button (marked "Zero") is slid to the left.

Blank Control Knob

This control is the knob on the upper right-hand side of the control panel marked "100% T."
It adjusts the needle on the meter to 100 percent transmittance (zero absorbance) when the cuvette is filled with distilled water or blank solution.

Shutter Button

This control, which is labeled "Zero," is located below the meter.
Sliding the button to the left cuts light off from the phototube and so permits the meter to be set to zero percent transmittance.

Wavelength Selector

This control, which is a knurled wheel located at the right-hand edge of the wavelength scale, is rotated to select the wavelength.
Wavelength scale: Wavelengths in the visual spectral range of 400 to 650 mμ can be selected. The indicator is moved to the line marked "Aux. Fltr." (Auxiliary Filter), which is located toward the right-hand edge of the wavelength scale, when a standard auxiliary filter is employed.

Suction Button

This button is located in the lower left-hand corner of the front of the instrument, just to the left of the cuvette assembly. It is pressed to aspirate sample and cleaning solutions from the cuvette.

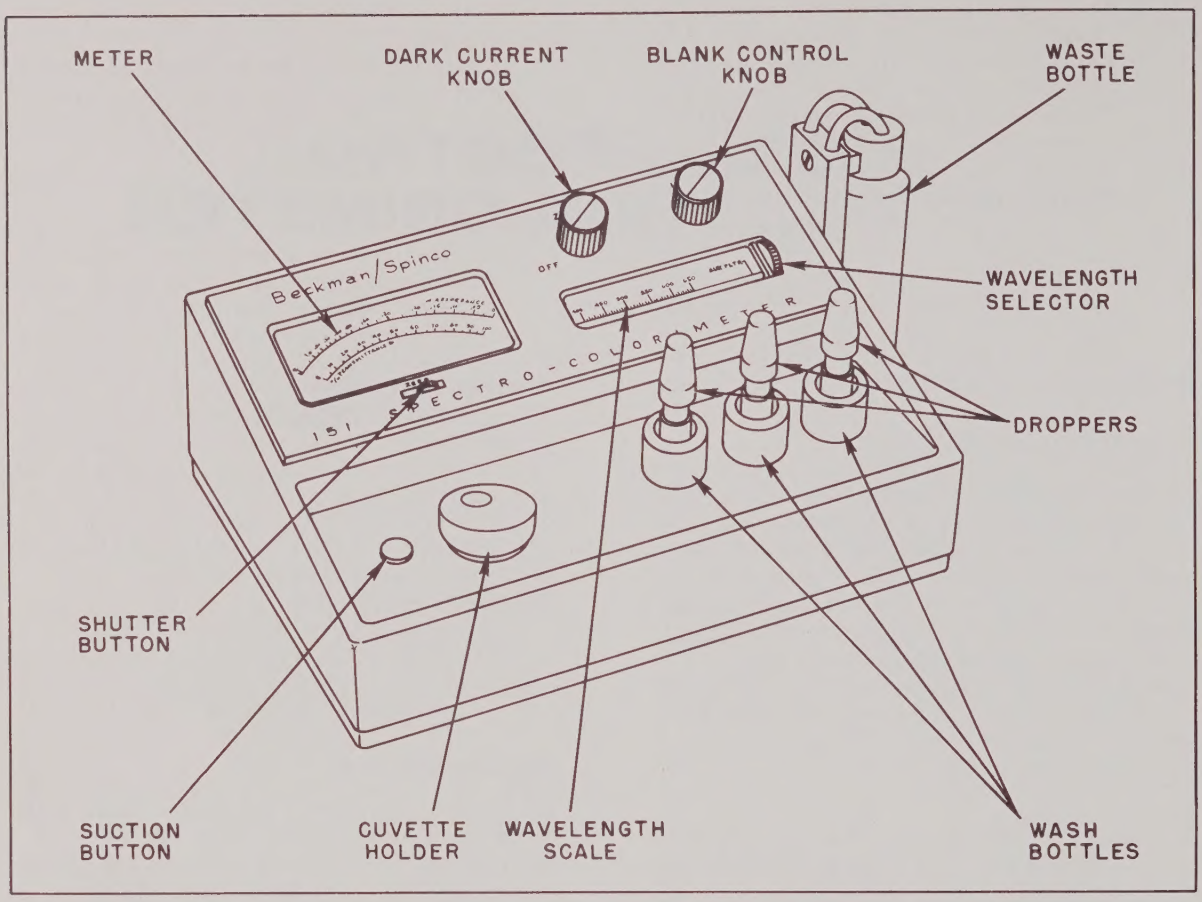

Figure 1. Location of Parts and Controls, Beckman Model 151 Spectro-Colorimeter.

Meter

Readings are indicated directly on the meter. The needle points to these two scales simultaneously:

(1) absorbance, which reads from 0 to infinity; and

(2) percent transmittance, which reads from 0% to 100%.

OPERATION

Operating Procedure

WARNING: *Do not tip Spectro-Colorimeter onto its side or back without first removing the waste and wash bottles. Otherwise, liquid waste will run into the pump and disable it.*

A. Turn the Instrument on

1. Plug the power cord into the power outlet of the type specified on the name plate.
2. Rotate the Dark Current knob (marked "Off/Zero") clockwise to switch the instrument on. (*See Figure 3.*) Allow ten minutes for the instrument to warm up.

Note: The instrument may be left on continuously without damage.

B. Select Bandwidth

Select the optimum wavelength for the particular analysis. This wavelength is specified in the instructions for the analysis.

In selecting the wavelength on the instrument, use EITHER:—

1. Regular wavelengths

Rotate the wavelength selector to select the desired wavelength on the scale. *(See Figure 4.)*

OR, alternatively, use:—

2. Auxiliary wavelength-filters

If a standard auxiliary filter is to be used instead, employ the following procedure:

a. Rotate the wavelength selector until the indicator points to the line marked "Aux. Fltr." (Auxiliary Filter) at the right-hand edge of the wavelength scale. (*See Figure 5.*) This moves the glass filter from the light path.
b. Remove the cuvette holder from the instrument by pulling it straight up out of its socket. (*See Figure 6.*)
c. Wipe the standard auxiliary filter clean with optical tissue.
d. Insert this filter into the hole in the side of the cuvette holder, near the bottom. (*See Figure 7.*)
e. Replace the cuvette holder in the instrument.

C. Establish Reference

1. Transfer at least 100μl. of distilled water or blank solution into the cuvette. *(See Figure 8.)* It is important to prevent the formation of bubbles in the cuvette as they distort the optical path and cause inaccurate readings. To prevent bubble formation, insert the tip of the colorimeter pipette to the bottom of the cuvette and fill the cuvette slowly. Remove the pipette from the cuvette.
2. Slide the shutter button to the left to prevent the light beam from going through to the phototube. (*See Figure 9.*)
3. Keeping the shutter button pressed to the left, rotate the Dark Current (Off-Zero) knob to adjust the needle on the meter to read 0 percent transmittance. (*See Figure 10.*)
4. Release the shutter button, allowing it to slide back to the right.
5. Rotate the Blank control (100% T) knob to adjust the indicator on the meter to read 100 percent transmittance. (*See Figure 11.*) (*If a major adjustment of the Blank control has to be made, it may be necessary to recheck the zero adjustment of the percent transmittance scale: see paragraph 3 above.*)
6. Press the suction button to empty the cuvette. (*See Figure 12.*) Hold the button down for five seconds. The buzzing sound is normal and indicates the suction pump is working correctly. The meter needle should return to approximately the same setting each time the cuvette is emptied.

D. Record Reading

1. Transfer not less than 100μl. of the sample to be analyzed into the cuvette. (*See Figure 13.*) It is important to prevent the formation of bubbles in the cuvette as they distort the optical light path

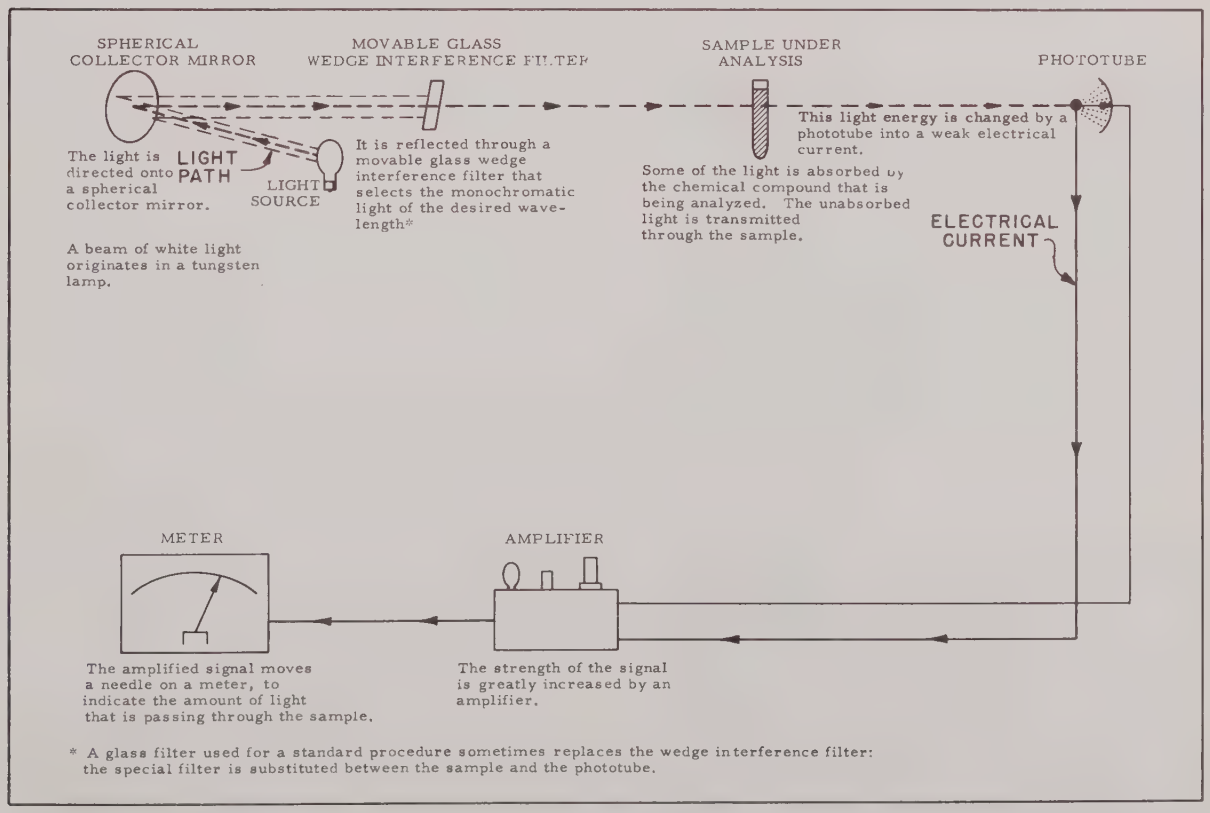

Figure 2. Light Path of Beckman Model 151 Spectro-Colorimeter.

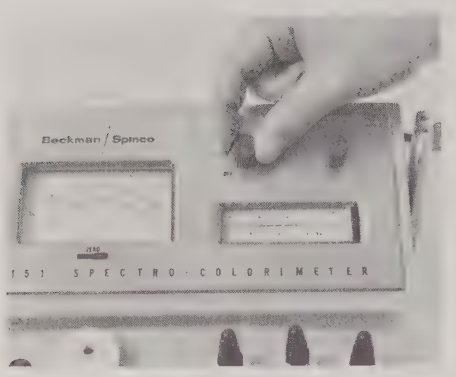

Figure 3. Turning on Dark Current Knob.

Figure 4. Rotating Wavelength Selector.

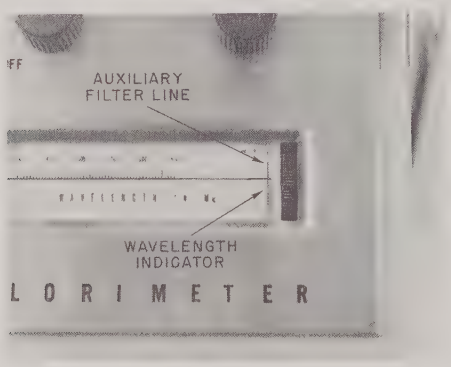

Figure 5. Indicator on Auxiliary Filter Line.

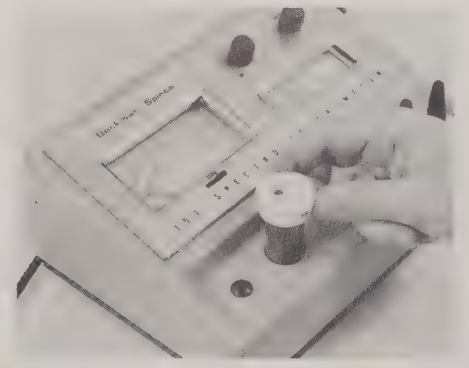

Figure 6. Removing Cuvette Holder.

and cause inaccurate readings. To prevent bubble formation, insert the tip of the colorimeter pipette to the bottom of the cuvette and fill the cuvette slowly. Remove the pipette from the cuvette.

2. Record the *direct* reading in percent transmittance or absorbance units on the meter.
3. Flush the cuvette by pressing the suction button for five seconds.
4. Other samples, in the same type of determination, can now be read and flushed out, in rapid sequence.
5. After completion of all readings in the determination, clean the cuvette as described below.

E. Clean Cuvette

Keep the cuvette clean to obtain the best results. The cuvette should be cleaned:

(i) after each 20 readings; and
(ii) when changing determinations (such as from bilirubin to albumin), or after the instrument has been unused for a long period.

The three wash bottles should be used to clean the cuvette as follows:

1. a. Put distilled water from the distilled water bottle into the cuvette with the dropper.
   b. Flush the cuvette by pressing the suction button.
2. a. Put 1% detergent solution into the cuvette.
   b. Flush the cuvette.
3. a. Put distilled water into the cuvette again.
   b. Flush the cuvette.
4. a. Put 1% caprylic alcohol in acetone into the cuvette.
   b. Flush the cuvette.
5. Leave the cuvette filled with distilled water until the next time it is used.

F. Other Determinations

Different determinations may be done after the selection of wavelength and establishment of a reference for each.

Operating Precautions

Sample Disposal System

1. Make certain that the waste bottle is screwed on tight to insure a good draw on the cuvette.
2. The waste bottle can be emptied after it has been unscrewed.

Wavelength Selector

CAUTION: Do not move the needle to the left of 400, or to the right of the line marked "Aux. Fltr.", on the wavelength scale or you will jam the mechanism.

MAINTENANCE

Trouble Shooting

SYMPTOM	REMEDY
Sample Disposal System	
Pump is not drawing.	Check for loose tube.
Pump is drawing inadequately or sluggishly.	Adjust armature nut on pump.
Meter Needle	
Needle does not move off zero at any time.	Fuse is blown.
Needle can be zeroed by Dark Current control, but needle does not move at all when shutter button is released.	Lamp bulb is burned out. Replace bulb and re-focus mirror by turning the three screws to obtain maximum indication of light transmittance.
Wavelength Selector	
(i) Mechanism jams.	(i) Remove top of instrument as described in "Routine Maintenance" below. (ii) Put leading wires back onto threads of mechanism screw. (iii) Replace top.

Routine Maintenance

Always remove waste and wash bottles before tipping instrument.

Fuse Replacement

The instrument has only one fuse. It is attached to the lamp holder assembly. A spare fuse is supplied. Replace a blown fuse in the following way:

1. Remove the access plate, located underneath the instrument.
2. Unscrew and remove the fuse screw-cap and the blown fuse.
3. Attach the new fuse to the screw-cap.
4. Replace the screw-cap.
5. Replace the access plate.

Removal of Top

Remove the Spectro-Colorimeter top as follows:

1. Remove the cuvette holder from the instrument by pulling it straight up out of its socket.
2. Remove the three wash bottles and their droppers.
3. Remove two screws from the side of the Dark

Figure 7. Inserting Filter.

Figure 8. Transferring Blank Into Cuvette.

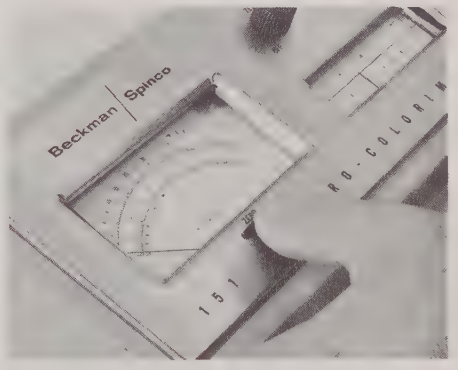

Figure 9. Sliding Shutter Button to Left.

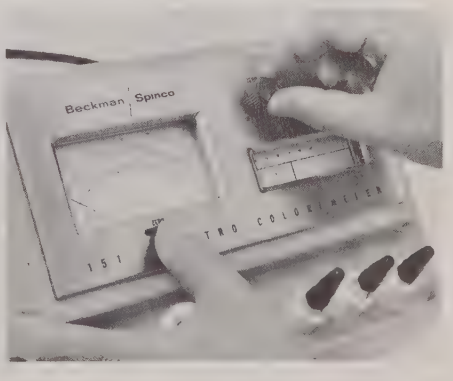

Figure 10. Shutter Button to the Left, Rotating Dark Current Knob.

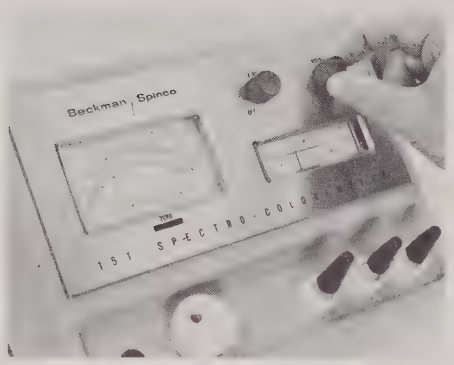

Figure 11. Rotating Blank Control Knob.

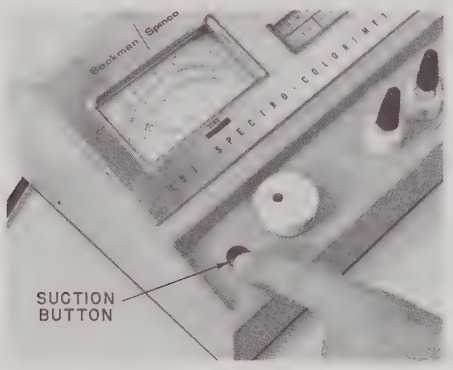

Figure 12. Pressing Suction Button.

Figure 13. Transferring Sample Into Cuvette.

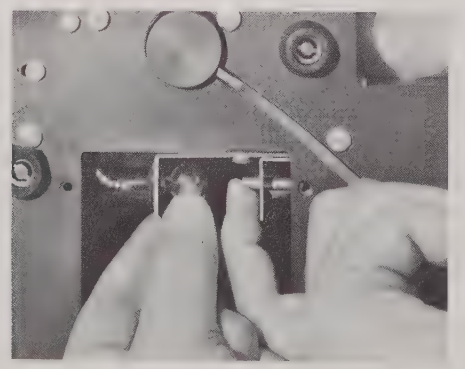

Figure 14. Pushing Rod Out of Way of Bulb.

Current Knob, then remove the knob.

4. Remove two screws from the side of the Blank Control Knob, then remove the knob.
5. Remove the four fillister head screws underneath the instrument.
6. Lift the top straight up to remove it from the instrument.

Lamp

Replace a burned-out lamp as follows:

1. Remove the access plate.
2. Push the spring-loaded rod out of the way of the lamp. *(See Figure 14.)*
3. Pull the lamp out of its socket.
4. Insert the new lamp into the socket. Note that the pins in the lamp go correctly into their holes in the socket when the filament is vertical.
5. Focus mirror if necessary.
6. Replace access plate.

Focusing the Mirror

After replacing a lamp, the mirror focus must be checked:

1. Set the wavelength selector to 650 mμ.
2. Remove the waste and wash bottles.
3. Turn the instrument onto its left side, with the bottom facing toward you.
4. Remove access plate.
5. Turn the instrument on. The light beam reflected by the mirror should strike the *edge* of the hole behind the lamp, as shown in Figure 15. If the beam is in this position, set the meter to about 3/4 full scale, and adjust *one* of the outside adjusting nuts *and* the inner adjusting nut until the meter needle peaks. Or, in other words, adjust the mirror for maximum light transmission. The nut adjustments will in all probability be slight—seldom more than 1½ turns.

   If, upon turning the instrument on, the light beam is not in the position indicated, adjust all three nuts until it is. Then adjust two nuts for maximum light transmission, as described above.

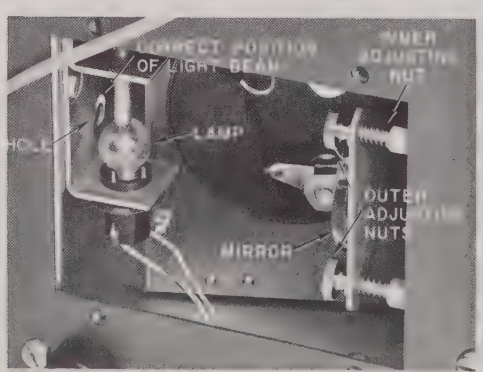

Figure 15. Lamp, Mirror, and Correct Position of Light Beam.

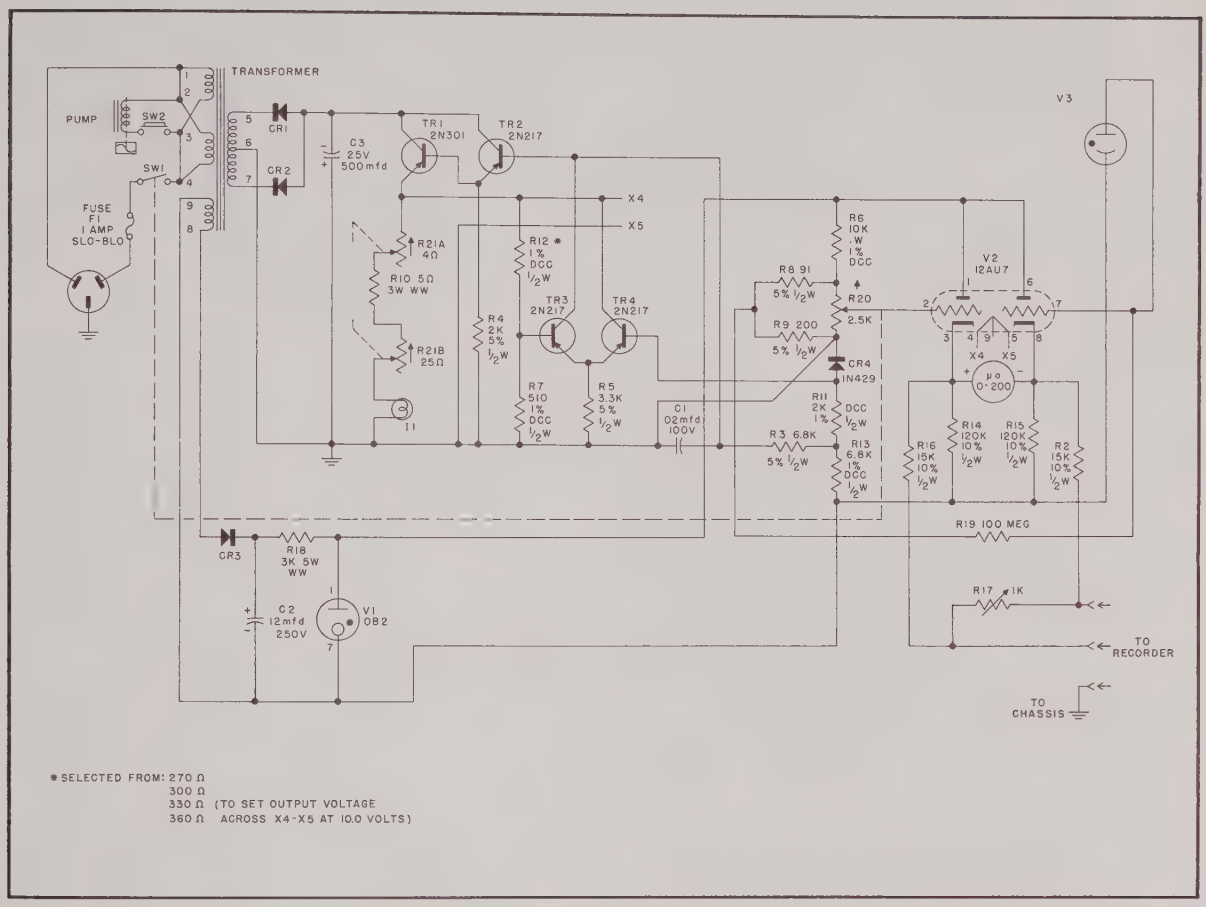

Figure 16. Spectro-Colorimeter, Beckman Model 151, Schematic Diagram.

Replacement Parts List

No.	Item
314274	Polyethylene Rinsing Pipette *(for colorimeter wash bottles)*
315232	Phototube Assembly
092237	Vacuum Tube, OB2
800256	Vacuum Tube, 5963
811603	Waste Bottle *(glass)*
314526	Colorimeter Pipette *(for inserting sample into cuvette, Pkg. of 10)*
314181	Cuvette Assembly
315245	Macrocuvette Adapter *(accepts 19 x 105 mm round and 13 x 13 mm square cuvettes)*
315246	Macrocuvette Adapter *(accepts 19 x 150 mm round cuvettes)*
315247	Macrocuvette Adapter *accepts 12 x 75 mm round and 8 x 8 mm square cuvettes)*
315348	Macrocuvette Adapter *(accepts 10 x 75 mm round cuvettes)*
314206	Pump Assembly
807588	Lamp
000148	Fuse

Technical Bulletin No. 6059C

Beckman Model 150 Ultramicro Analytical System MICROFUGE

INTRODUCTION

An advantage of the Beckman Model 152 Microfuge *(See Figure 1)* is that this high-force microcentrifuge carries out centrifugation speedily. Due to its instant acceleration and deceleration, the instrument centrifuges each small-volume samples in a fraction of the time usually required.

The rotor is driven by a premium-quality series-wound motor of fractional horsepower. The same rotor is used at all times. Variable speed is not necessary since sedimentation is controlled by the duration of the centrifugation run. Four vertical slots, in opposing pairs, are machined in the rotor walls. A metal slide fits into each slot. Five holes are punched in each slide. Micro test tubes fit into the holes to permit centrifugation and each tube is held in the slide by its flared lip. Any number of samples up to 20 may be centrifuged at one time. Each slide is labeled with an identification letter (A, B, C or D) and each sample is identified by its position on a particular slide.

Each micro test tube used to collect blood has a capacity of 400μl. The micro test tubes are made of polyethylene so no sample is lost by wetting of surfaces. The tubes are thrown away after use.

A tube rack is supplied with each Microfuge. The rack contains numbered holes into which the micro test tubes fit.

OPERATING PROCEDURE

1. Fill Tube

a. Fill a micro test tube with sample. Follow the procedure described in the "Micropipette" instructions. A flick of the wrist will remove any air bubbles trapped underneath the surface.
b. Fill other tubes.

2. Load Slides

a. Load filled tubes into a slide (*See Figure* 2). Push tubes as far through the holes as they will go.
b. Note the position of each sample, to permit subsequent identification.
c. After the slide has been loaded, place its filled tubes in the appropriate holes in a tube rack until the tubes are ready for centrifugation.
d. Load further slides as necessary. (*See* 3.*c*, *on balancing load*, *below*.)
e. Place tubes of other loaded slides in holes in the tube rack.

3. Load Rotor

a. Remove the cover from the centrifuge.
b. Insert the first loaded slide into a slot in the rotor. (*See Figure* 3.) Push the slide as far down as it will go, so that its top does not scratch the cover.
c. Balance the load: add slides loaded with tubes of water, as necessary, so that opposing slides carry an equal number of tubes.

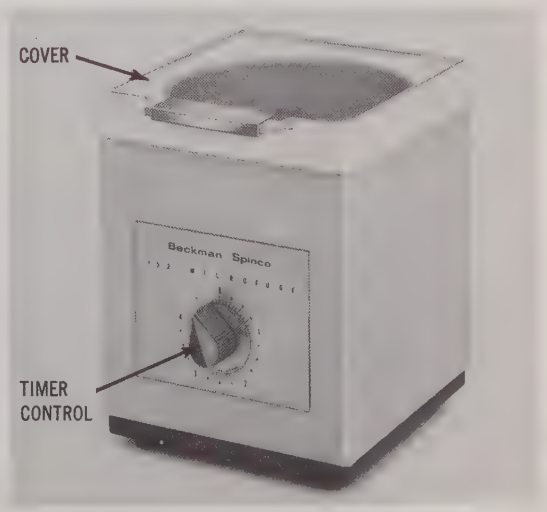

Figure 1. Beckman Model 152 Microfuge.

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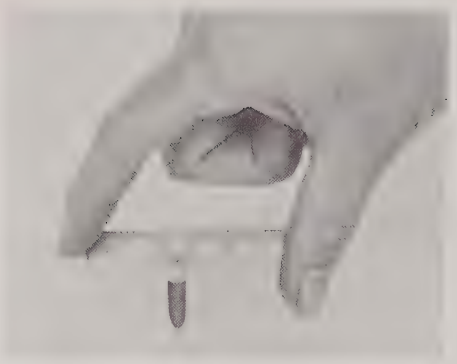

Figure 2. Tube in Slide.

Figure 3. Rotor Loaded.

Figure 4. Closing Cover.

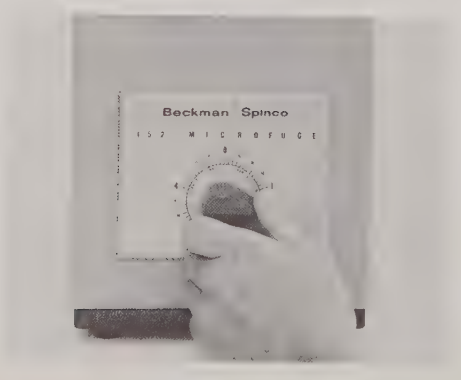

Figure 5. Turning Timer Control Past One.

4. Centrifuge Load

a. Close the centrifuge cover. *(See Figure 4.)* This is a safety precaution.

b. Turn the Timer control past the figure 1 on the dial. *(See Figure 5.) Do this even though the desired length of run is less than one minute.* This step is necessary to insure accurate timing. Rotor automatically starts to spin at its fixed speed when the Timer control is rotated clockwise.

c. Turn the Timer control to the length of run recommended in instructions for the particular analysis. If the selected run time is *less than* one minute, turn the Timer control back to the desired number of seconds. *(See Figure 6.)*

(Note: The centrifuge may be stopped at any time by turning the Timer control to 0.)

5. Unload Rotor

The centrifuge automatically turns itself off at the completion of a run of selected period. The samples are now ready for analysis.

Note: The Microfuge has been designed on the assumption that the motor will be rested between runs (long enough to permit unloading and loading), to prevent overheating.

MAINTENANCE

Routine Maintenance

Store in a dust-free container.

Clean out the rotor chamber by means of a damp sponge. A detergent, such as Alconox, may be used if the sponge does not clean effectively.

Trouble Shooting

SYMPTOM	CHECK
(i) Excessive vibration. (ii) Centrifugation not completed within selected period.	In either case, check that: (i) load is properly balanced. (ii) cover is in position. (iii) Timer is properly set.
Motor does not start.	(i) Make certain that electrical connection is plugged in and timer is turned on.

Figure 6. Turning Timer Control Back.

Technical Bulletin No. 6060C

Beckman Model 150 Ultramicro Analytical System MICROTITRATOR

INTRODUCTION

With the Beckman Model 153 Microtitrator, a series of titrimetric determinations can be performed rapidly on very small volumes. The volume of titrant used is indicated on the dial (*see Figure 1*). From the dial reading, the concentration of the substance being analyzed can be calculated. The dial can be zeroed after each determination in order to obtain direct readings.

The Model 153 multi-cup turntable accommodates 10 disposable 500μl titration cups. As an added convenience, a stable color reference may be placed in the center of the turntable to serve as a representative end point for the samples. The black and white turntables provide a choice of background color.

Since small volumes are employed, a series of samples can be titrated without refilling the burette. A rod in the micrometer-driven burette slides through a bushing and forces titrant out of the delivery tip. When the collar is removed, the burette tip may be detached for easy cleaning.

The burette assembly and an adjustable stirrer are attached to the burette mount. During titration, the vibrator-type stirrer mixes the sample. The electromagnetic stirrer motor is located within the burette mount.

FUNCTION OF CONTROLS

Micrometer Screw

Clockwise rotation of the micrometer screw drives the rod into the burette reservoir and forces titrant out of the delivery tip. The pointers on the dial rotate with the micrometer screw, thus indicating the volume of titrant expelled.

Dial Rotation Screw

The dial rotation screw is mounted behind the dial. This screw may be loosened in order to position the dial for special purposes. For example, the dial may be rotated 90 degrees for convenient operation by a left-handed person.

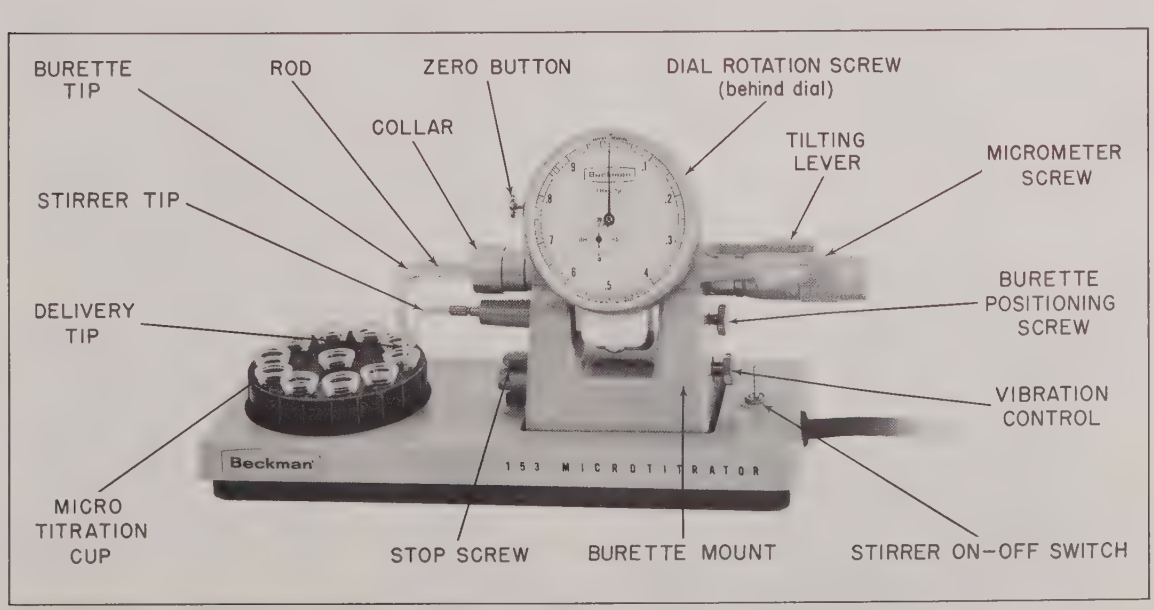

Figure 1. Location of Components and Controls.

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Zero Button

The zero button zeros the pointers on the dial.

Stop Screw

Rotation of the stop screw adjusts the vertical height of the stirrer tip and the burette delivery tip.

Stirrer On-Off Switch

The stirrer on-off switch actuates the stirrer motor.

Vibration Control

Rotation of the vibration control screw adjusts the intensity of stirring.

Burette Positioning Screw

When the burette positioning screw is loosened, the burette can be rotated about a horizontal axis or moved laterally. This permits precise positioning of the burette delivery tip.

Tilting Lever

When the tilting lever is pressed down, the burette delivery tip and the stirrer tip are raised clear of the micro titration cup.

NOTE: To keep the burette raised without continuing to press the tilting lever down, place an inverted micro titration cup underneath the stop screw.

OPERATING PROCEDURE

The following generalized procedure describes the basic steps of a typical titration run. However, since specific details may vary from one analysis to another, the user should follow the instructions which describe each particular application.

A. Fill Burette Reservoir

1. Remove Burette Tip
   a. Unscrew the collar that holds the burette tip in position.
   b. Remove the collar and the burette tip.

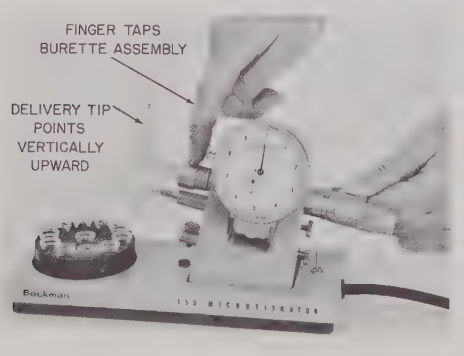

Figure 2. Removing Air Bubbles.

2. Fill Burette Reservoir
   a. Rinse the delivery tip with distilled water. Carefully wipe the tip dry.
   b. Slip the aspirator bulb over the mouth of the burette reservoir. Dip the delivery tip into the titrant supply and draw the liquid into the reservoir. When the burette reservoir is full, remove the aspirator bulb and turn the delivery tip up to prevent loss of titrant.

3. Replace Burette Tip
   a. Use a clean dry tissue to wipe off any solution clinging to the rod.
   b. Rotate the micrometer screw counterclockwise until the rod is withdrawn as far inside the burette cylinder as it will go.
   c. Hold the delivery tip upward so that a convexity of fluid forms at the mouth of the reservoir. This minimizes the formation of air bubbles when replacing the burette tip.
   d. Put the filled burette tip back into position with the delivery tip pointing vertically upward. Some liquid will be forced out of the delivery tip.
   e. Replace and tighten the collar.

4. Remove Air Bubbles
   a. Tilt the burette mount by pressing down on the tilting lever so that the burette delivery tip points further into the air. (*See Figure 2.*)
   b. Tap the burette assembly to expel any air bubbles through the delivery tip. To force out air bubbles, rotate the micrometer screw clockwise. All bubbles must be removed.

5. Prepare to Titrate
   a. Loosen the collar slightly. Press down on the tilting lever to lift the burette tip. Rotate the burette tip until the delivery tip points downward; then tighten the collar.
   b. Rotate the micrometer screw clockwise until the level of the liquid in the burette tip is precisely flush with the mouth of the delivery tip.
   c. Place up to 10 titration cups in the multi-cup turntable.

B. Titrate Standard

1. Fill Titration Cup

The method used to fill and empty pipettes is described in the Micropipette instructions.

   a. Pipette the desired amount of standard from the standard Micropipette into the titration cup.
   b. Pipette the desired amount of reagent from the reagent Micropipette into the titration cup.
   c. Pipette the desired amount of indicator from the indicator Micropipette into the titration cup.

2. Position Delivery Tip and Stirrer Tip
   a. Rinse the stirrer tip with distilled water. Carefully wipe the tip dry.
   b. Place the delivery tip and the stirrer tip below the surface of the liquid in the titration cup. This is to prevent splashing and formation of air bubbles during stirring. Optimum placement below the surface must be learned by trial and error for each titrimetric analysis. To adjust the vertical height of the delivery tip and the stirrer, rotate the stop screw.
   c. Adjust the burette positioning screw so that the delivery tip does not touch the sides of the titration cup or the stirrer.

3. Set Controls
   a. Turn on the stirrer.
   b. Rotate the vibration control screw to obtain the desired intensity of stirring.
   c. Press the zero button on the side of the dial to zero the pointers.

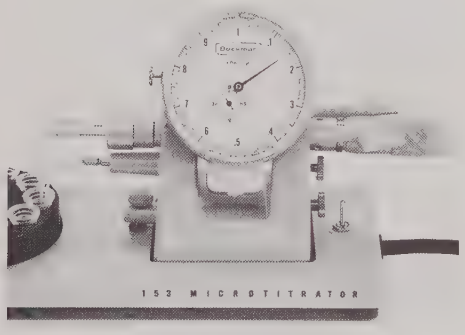

Figure 3. Dial Reading.

4. Titrate
   a. Rotate the micrometer screw clockwise slowly.
   b. As soon as the solution produces the most reproducible end point, stop rotating the micrometer screw.
   c. Record the dial reading. (*See Figure 3.*) This reading is a measurement of the volume of titrant that was expelled into the titration cup. One revolution of the large pointer corresponds to 1µl. The small dial records complete revolutions of the large pointer and reads directly in microliters.

5. Prepare Color Reference (Optional)

If desired, a standard that has a stable end point can be placed in the center of the multi-cup turntable to serve as a color reference for the titration series. To prepare a color reference, titrate several standards and choose one as representative of the proper end point. Place the color reference in the center of the turntable and duplicate its color as nearly as possible for all the samples in the series. Prepare a color reference before beginning each titrimetric series.

C. Titrate Sample

1. Series of Titrations

Use the following procedure for each sample in the series.

   a. Pipette the desired amount of sample from the sample Micropipette into the titration cup.
   b. Except for the first step of pipetting sample instead of standard, follow directions 1 through 4 under heading "B. TITRATE STANDARD." Flush the delivery tip before each titration by expelling a few drops of titrant onto a tissue. Then wipe the tip dry and zero the dial.
   c. If using a color reference, stop titration when the end point of the sample is the same as the reference end point.

      NOTE: If the titrations are made in succession and without interruption, it generally should not be necessary to titrate another color reference.

   d. After recording the dial reading for each sample, calculate the concentration of the unknown in each sample by dividing the reading of the sample by the reading of the standard and then multiplying by the concentration of the standard.
   e. To position each titration cup, press down on the tilting lever to raise the burette tip and then rotate the turntable until the succeeding cup is directly under the delivery tip. Make sure the detents in the turntable fit into the positioning notches in the base.
   f. Always zero the pointers on the dial after each titration.
   g. Discard titration cups after use.
   h. To prevent formation of air bubbles when titrant remains in the burette tip for prolonged periods or overnight, immerse the delivery tip in titrant or turn the tip vertically upward.

2. Refilling the Burette Reservoir

To refill the burette reservoir after the initial filling, dip the delivery tip into the supply of titrant and rotate the micrometer screw counterclockwise so that liquid flows up into the burette tip. If it is necessary to refill the burette reservoir while titrating a series of samples, always refill from the same bottle of titrant used for the standard.

D. Changing Titrants

Before changing to another titrant, remove the burette tip and thoroughly rinse it and the delivery tip. Place a receptacle under the burette cylinder and rinse the rod and the cylinder with distilled water. Wipe the rod and cylinder dry. Then fill the burette reservoir.

MAINTENANCE

A. Cleaning Burette Tip

Normally, rinsing the burette tip with distilled water will clean it adequately. However, if a film forms in the burette reservoir, use the following procedure to clean the burette tip.

1. Unscrew the collar and remove the burette tip.
2. Soak the burette tip and the delivery tip in a suitable cleaning solution such as Alconox. Rinse both with distilled water.
3. If the burette tip or the delivery tip is blocked, a wire may be used to clean out particles. Care must be taken not to break or chip the burette tip.
4. Replace the burette tip and tighten the collar.

B. Care of Microtitrator

1. To prevent damage to delivery tip and stirrer tip:
   a. Always adjust the stop screw so the delivery tip and stirrer tip do not touch the bottom or sides of the titration cups.
   b. Avoid bending the delivery tip. Before rotating the turntable or moving the burette tip, press the tilting lever to raise the delivery tip and stirrer tip.
2. Do not use the dial as a handle when moving or lifting the Microtitrator. Lift the instrument by its base or the burette mount.

Technical Bulletin No. 6061C

Beckman Model 150 Ultramicro Analytical System MICROMIXER

INTRODUCTION

Precise analysis in ultramicro work depends upon several factors, including the thorough mixing of solutions when mixing is prescribed. By means of vibration, the Beckman/Spinco Model 154 Micromixer automatically mixes the contents of up to five micro test tubes.

Power requirements for the Model 154 Micromixer are 115 volts at either 50 or 60 cycles. On 60-cycle instruments, the timing scale ranges from 0 to 60 seconds; on 50-cycle instruments, from 0 to 50 seconds.

FUNCTION OF CONTROLS

(*See Figure 1. and Figure 2.*)

The Vibrator

The vibrator, made of white linear polyethylene, is mounted on the top of the instrument case. A horizontal slot in the vibrator holds the tube holder firmly in place during mixing. To provide for the mixing of hand-held tubes, the vibrator is grooved along the back at its outer edge. The groove is fitted to the shape of the micro test tubes.

The Vibrator Control Screw

The vibrator control screw adjusts the amplitude of vibration. Before shipment the Micromixer is adjusted for the maximum amplitude of vibration. The user, however, may adjust the amplitude according to his preference.

The Pointer

The pointer indicates the duration of mixing on the timing scale.

The Set Knob

The set knob rotates to adjust the pointer and selects the number of seconds desired for each mixing.

The Actuating Button

The actuating button, located in the center of the pointer, operates the Micromixer for all time-regulated mixings. It turns the Micromixer off *automatically* at the end of the selected time interval.

The On-Off Button

When the contents of several tubes are to be mixed individually, the on-off button can be used to turn the Micromixer on for an indefinite period of time. It *does not* automatically turn the mixer off.

CAUTION: ***Do not leave the Micromixer running unless it is in use.***

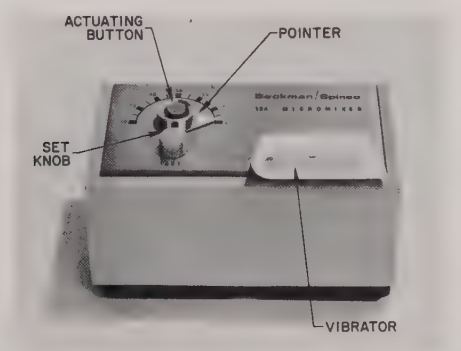

Figure 1. Front of Micromixer.

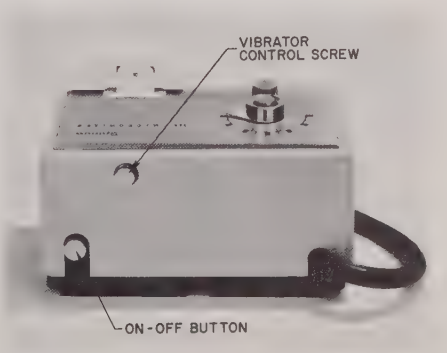

Figure 2. Back of Micromixer.

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OPERATING PROCEDURE

Time-Regulated Mixing

Because a wide variety of solutions will be mixed, it is impractical to recommend a specific time interval for all mixing. Generally, 15 seconds mixing time is sufficient.

1. Securely fasten the caps on the tubes whose contents are to be mixed.
2. Put the tubes in the tube holder.
3. Insert the tube holder in the horizontal slot of the vibrator, short end first, as shown in *Figure 3*.
4. Using the set knob, select the desired time interval for the mixing.
5. Press the actuating button.
6. At the conclusion of the mixing the Micromixer is automatically turned off.
7. If further mixing is required, repeat the process.

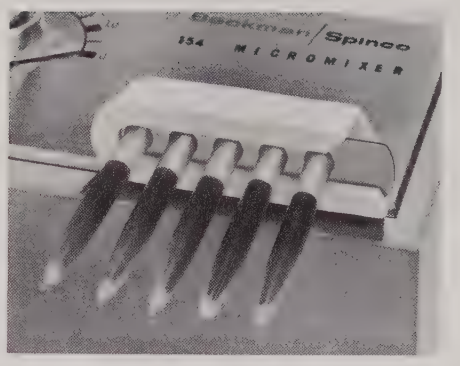

Figure 3. Time-Regulated Mixing.

Mixing of Hand-held Tubes

1. Cap the tube.
2. Using the on-off button, turn the Micromixer on.
3. Hold the tube between the thumb and the index finger as shown in *Figure 4*, with the index finger farther down the tube.
4. Pressing the tube against the vibrator with the index finger, slide the tube back and forth along the groove until the contents are thoroughly mixed.
5. At the conclusion of the mixings, turn the Micromixer off.

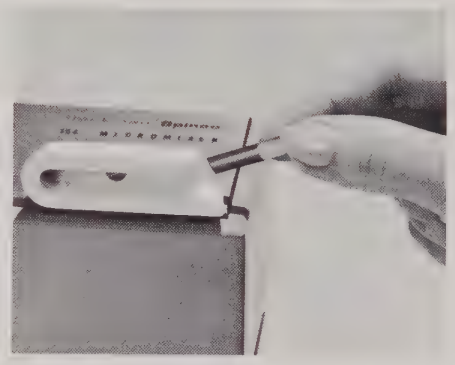

Figure 4. Mixing of Hand-held Tube.

Beckman/Spinco Model 150 Ultramicro Analytical System MICROPIPETTE

INTRODUCTION

The Beckman/Spinco Micropipette delivers a constant, accurate volume of either (a) sample, or (b) reagent. The sample Micropipettes (*See Figure 1*) are identical to the reagent Micropipettes (*See Figure 2*) except that, since the sample pipette picks up sample from an outside source, the stem inside its bottle is shorter.

Each Micropipette measures a predetermined volume of sample or reagent. A variety of different volumes of pipettes is offered: sample and reagent pipettes of the correct volume for each procedure are supplied with each appropriate Micropack. Both sample and reagent are delivered by the volume of the pipette used and the volume is stated on the Micropipette label. *Always pipette sample and reagent with maximum accuracy: accuracy of the final result depends on this.*

USING SAMPLE (SHORT STEM) MICROPIPETTE

How to Fill

(*See Figure 3.*)

1. Insert Pipette Into Sample
   a. Grasp the bottle between thumb and middle fingers.
   b. *Gently* squeeze the sides of the bottle between thumb and middle fingers.
   c. Place forefinger over the hole in top of the dome which is on top of the bottle.
   d. Insert the tip of the pipette below the surface of the sample in its vessel. Be certain *not* to release the pressure of thumb and middle fingers as the pipette is inserted.

2. Fill Pipette
   a. *Very slowly* release the pressure of thumb and middle fingers on the sides of the bottle. (*Do not, however, release the pressure of the forefinger over the hole in the dome.*)
   b. Observe the sample move *slowly* up the inside of the pipette, traveling all the way from its delivery tip to its inner end which is inside the dome.
   c. Allow one drop of sample to overflow from the inner end of the pipette; this automatically washes out the pipette and insures that a constant, accurately measured volume is obtained.
   d. Make certain that no air bubbles are present in the sample in the pipette.

3. Remove Pipette
   a. Remove the forefinger from its position over the hole in the top of the dome.
   b. Remove the tip of the pipette from the supply of sample.
   c. Remove any remaining pressure of thumb and middle fingers against the sides of the bottle.
   d. Carefully wipe the tip of the pipette dry.

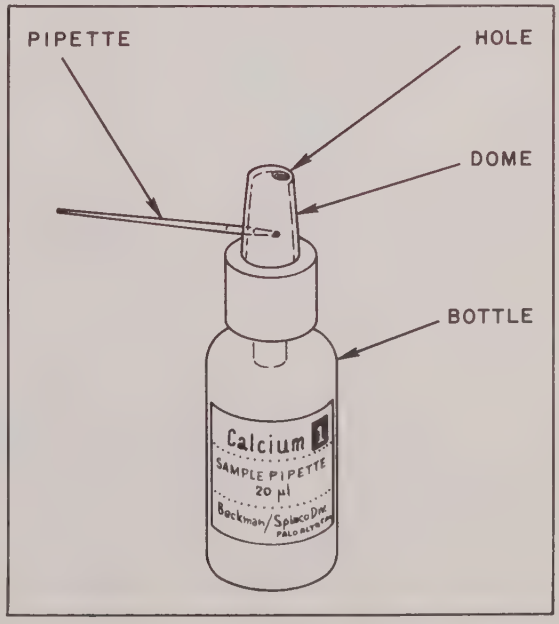

Figure 1. Sample Pipette.

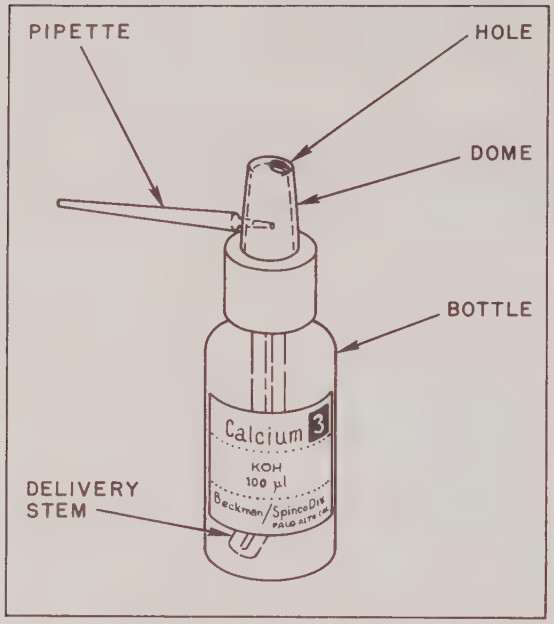

Figure 2. Reagent Pipette.

How to Deliver

(See Figure 4.)

1. Grasp Bottle
   a. Pick up the bottle between thumb and middle fingers—but do *not* yet apply any pressure against the sides of the bottle.
   b. Place forefinger over the hole in top of the dome which is on top of the bottle.

2. Deliver Sample
   a. Place the tip of the pipette against one inner side of the vessel into which the sample is to be delivered.
   b. *Slowly* expel the sample from the pipette into the vessel by *gently* squeezing the sides of the bottle, between thumb and middle fingers, until all the sample has been delivered. *It is particularly important to empty the pipette SLOWLY in order to obtain the best results.* Leave no residual liquid inside the pipette.
   c. Remove the pipette from contact with the vessel into which the sample has been delivered.
   d. Release any remaining pressure of thumb and middle fingers against the sides of the bottle.

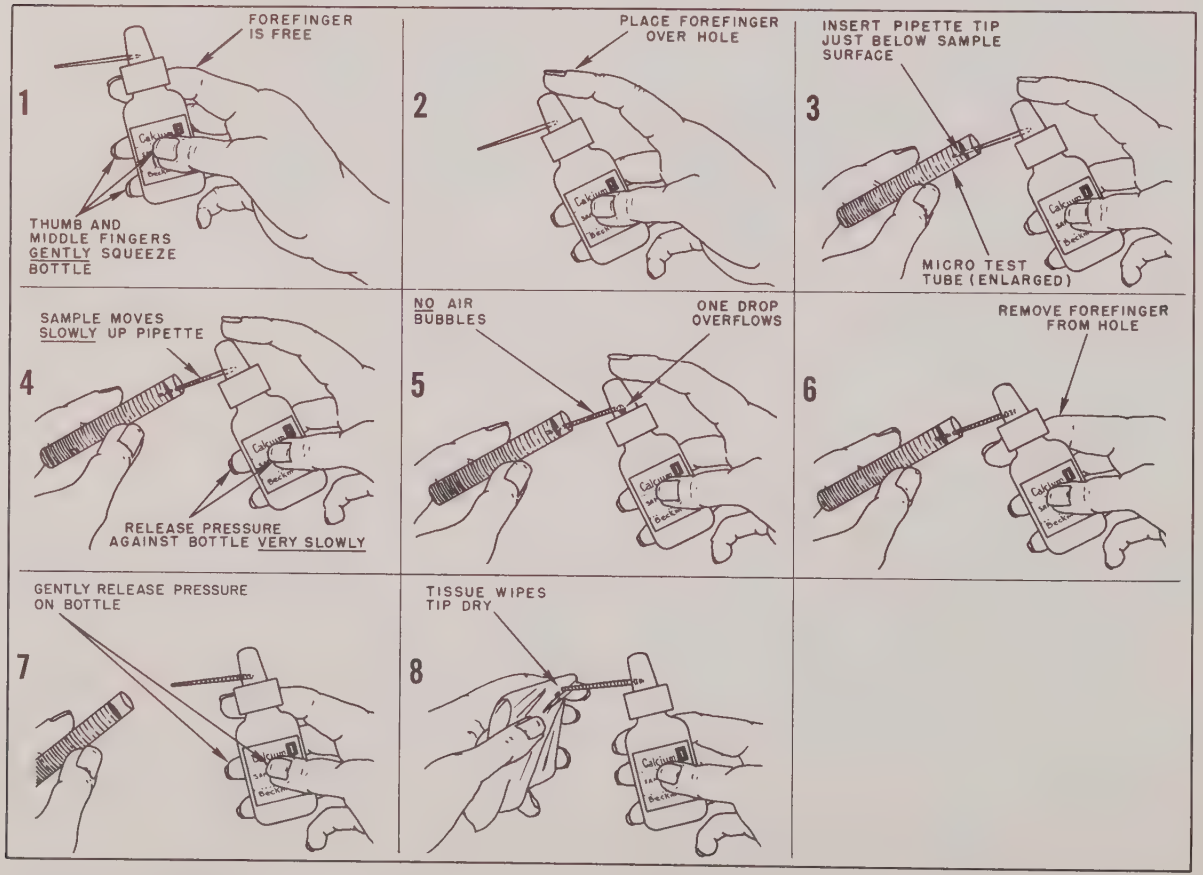

Figure 3. Filling Sample Pipette.

USING REAGENT (FULL-LENGTH STEM) MICROPIPETTE

How to Fill

(*See Figure 5.*)

1. Check That Bottle is Full

At the beginning of each day's work, routinely check that sufficient volume of each reagent solution is available for the day's analyses.

2. Squeeze Bottle
   a. Grasp the sides of the bottle between thumb and middle fingers. Do *not*, at this time, place the forefinger over the hole in the top of the dome.
   b. Squeeze the sides of the bottle, between thumb and middle fingers, until the reagent solution rises into the dome to a level which is *above* the inner end of the pipette. (As a safety precaution, be careful *not* to squeeze the bottle so hard that solution is forced out of the hole in the top of the dome.)

3. Fill Pipette
   a. Place the forefinger over the hole in the top of the dome.
   b. Continue to squeeze the sides of the bottle gently, between thumb and middle fingers, until the pipette fills completely with reagent solution and one drop overflows from the delivery tip.
   c. Remove forefinger from its position over the hole in top of the dome.
   d. Release pressure of thumb and middle fingers from the sides of the bottle.
   e. Carefully wipe off the overflow drop.
   f. An accurately measured volume of reagent solution is now in the pipette, ready for delivery.

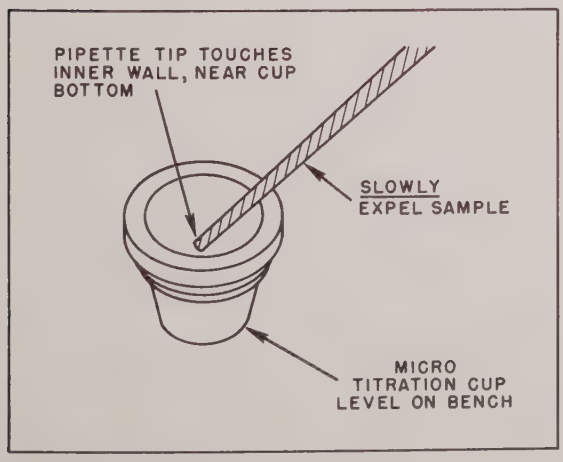

Figure 4. Delivery from Pipette.

How to Deliver

Follow the procedure described under the heading "How to Deliver" in the "Sample Micropipette" section and illustrated in Figure 3.

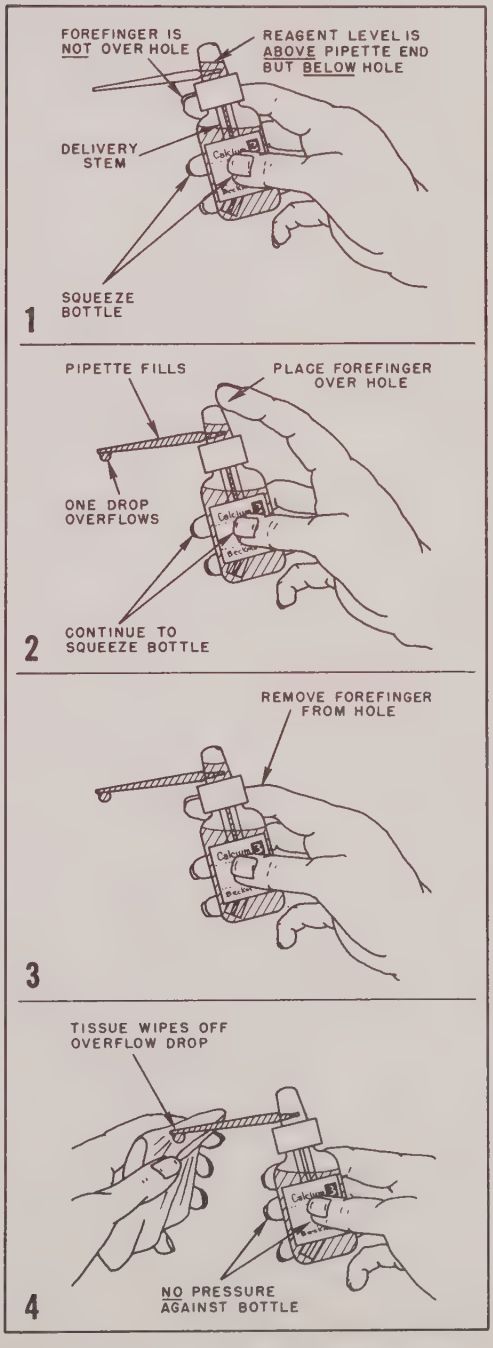

Figure 5. Filling Reagent Pipette.

MAINTENANCE-CLEANING

Sample Micropipette

1. Routinely rinse pipette with distilled water (a) after about every 20 deliveries; and (b) when changing from one type of determination to another, e.g., from calcium to total cholesterol.
2. An invisible film of protein forms on the inside of a pipette used to pipette serum, plasma and blood. To remove film, every evening fill the pipette with a fresh solution of 0.1N HCl which contains 0.5% pepsin. By morning, digestive action of the pepsin will have removed unseen film. Expel the cleansing solution and rinse.

Reagent Micropipette

Routinely rinse the reagent Micropipette before each day's work is begun.

Particles of dried-out reagent solution sometimes obstruct the pipette. This obstruction may be recognized by adhesion of droplets of solution to the inner walls of the pipette. Unblock the pipette as follows:

1. Force reagent solution from the bottle through the pipette.
2. If droplets remain, transfer the dirty pipette to a spare sample Micropipette (to prevent contamination of reagent solution in subsequent cleansing).
3. Fill the unclean pipette with a mixture of a detergent, such as Alconox, and distilled water. Leave for about one minute.
4. If droplets still do not disappear, fill the pipette with concentrated ammonium hydroxide. Allow it to stand for about 15 minutes. Repeat if necessary. (*One or two minute droplets will not usually affect accuracy.*)
5. If blockage prevents entry of detergent or ammonium hydroxide, soak the entire pipette in a bath of ammonium hydroxide or detergent solution.

Cleaning Precaution

Never clean an obstructed pipette with a stylette: such cleaning will scratch the inner surface and irreparably affect calibration.

Beckman Model 150 Ultramicro Analytical System

REPLACEMENT PARTS LIST

SUPPLIES

No.	Item
314766	Calcium Chempak
314768	Chloride Chempak
314767	Bilirubin Chempak
314770	Cholesterol Chempak
314769	Creatinine Chempak
314761	Glucose Chempak
314765	Phosphorus Chempak
314760	Total Protein Chempak
314764	Urea Nitrogen Chempak
314763	Uric Acid Chempak
314326	Micro Test Tubes *(Pkg. of 1000)*
314463	Micro Titration Cups *(Pkg. of 500)*

PARTS

Spectro-Colorimeter

314526	Colorimeter Pipette *(for inserting sample into cuvette, Pkg. of 10)*
314181	Cuvette Assembly
315245	Macrocuvette Adapter *(accepts 19 x 105 mm round and 13 x 13 mm square cuvettes)*
315246	Macrocuvette Adapter *(accepts 19 x 150 mm round cuvettes)*
315247	Macrocuvette Adapter *accepts 12 x 75 mm round and 8 x 8 mm square cuvettes)*
315348	Macrocuvette Adapter *(accepts 10 x 75 mm round cuvettes)*
314206	Pump Assembly
807588	Lamp
000148	Fuse
314274	Polyethylene Rinsing Pipette *(for colorimeter wash bottles)*
315232	Phototube Assembly
092237	Vacuum Tube, OB2
800256	Vacuum Tube, 5963
811603	Waste Bottle *(glass)*

Microfuge

314364	Test Tube Holders *(Pkg. of 8 lettered A thru H)* for holding Micro Test Tubes in Microfuge Rotor
314349	Dust Cover for Microfuge

Microtitrator

314437	Burette Tip Assembly *(Glass Reservoir plus Polyethylene Tip)*
314439	Burette Tip, polyethylene
314440	Disk Assembly *(white titration platform; holds one microtitration cup — for Microtitrators with serial numbers below 1500)*
315170	Microtitrator Conversion Kit *(converts one-cup-holder to ten-cup-holder Microtitrator)*
315117	Rotary Titration Cup Holder *(black, holds ten titration cups, for Microtitrators with serial numbers 1500 and above)*
315118	Rotary Titration Cup Holder *(white, holds ten titration cups, for Microtitrators with serial numbers 1500 and above)*
811605	Rubber Aspirator Bulb *(for filling microburette)*
314444	Stirrer Tip
314464	Titration Cup Rack *(holds 21 microtitration cups)*

Other

314510	Micropak Base
314511	Micropak Cover
314512	Test Tube Rack *(holds 40 micro test tubes)*

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Technical Bulletin 6064B

Method of Analysis with the Beckman Model 150 Ultramicro Analytical System

CHOLESTEROL

Ultramicro Adaptation of the Method of Zak

PRINCIPLE AND METHOD

Zak's method for the determination of total cholesterol: Zak, B. Am. J. Clin. Path. 27, 583 (1957). Adapted for ultramicro analysis by Jarnagin and Myers of the Spinco Division of Beckman Instruments, Inc.

Cholesterol is separated from proteins using a mixture of ferric chloride and glacial acetic acid. The addition of concentrated sulfuric acid to an aliquot of protein-free filtrate containing cholesterol, causes formation of a purple chromophore with an intensity proportional to the cholesterol concentration.

APPARATUS

400 μl micro test tubes
Cholesterol Micropak
  Sample Pipette: 5 μl
  Reagent Pipette: 200 μl
  Transfer Pipettes: 100 μl, 150 μl
Cholesterol Chempak
Beckman/Spinco Model 154 Micromixer
Beckman/Spinco Model 152 Microfuge
Beckman/Spinco Model 151 Spectro-Colorimeter
Water Bath: 100 ml beaker, hot-plate, thermometer

PROCEDURE

1. Transfer the Diluting and Precipitating Reagents from the glass bottles in the Chempak to their corresponding polyethylene bottles in the Micropak. At the end of the day, return the reagents to the glass bottles for storage.
2. Use 400 μl micro test tubes to prepare blank, standard, and unknown.
3. Into the "blank" tube, pipette:
   5 μl of distilled water
   200 μl of Diluting Reagent
   To prevent the reagent from leaking out, hold the pipette in a horizontal position.
4. Into the "standard" tube, pipette:
   5 μl of Standard
   200 μl of Diluting Reagent
5. Into the "unknown" tube, pipette:
   5 μl of serum
   200 μl of PRECIPITATING REAGENT
6. Cap the tubes and mix with the Micromixer.
7. Centrifuge the tubes for 30 seconds.
8. Transfer 150 μl of supernatant from the blank tube to a clean blank tube. Follow the same procedure with the standard and unknown.
9. Into the blank tube containing supernatant, carefully layer 100 μl of concentrated sulfuric acid. (Hold the test tube in a nearly horizontal position and pipette the sulfuric acid slowly into the tube near the lip.) TO AVOID CONTACT WITH THE ACID: place a small quantity of acid in a suitable container and use the transfer pipette for sulfuric acid to layer it into the tubes.
10. Cap the tube and mix completely with the Micromixer.
11. Insert the blank tube into a water bath that has been heated to approximately 80° C.
12. Leave the blank tube in the water bath for 5 minutes.

	Blank	Standard 200	Unknown
Distilled Water	5 μl.*		
Standard		5 μl.	
Serum			5 μl.
Diluting Reagent	200 μl.	200 μl.	
Precipitating Reagent			200 μl.
Cap and mix; centrifuge for 30 seconds			
Aliquot of Supernatant	150 μl.	150 μl.	150 μl.
Concentrated H_2SO_4	100 μl.	100 μl.	100 μl.
Mix then place in 80 C water bath for 5 minutes			

*The same pipette is used for blank, standard and unknown. Rinsing is done by allowing one drop of sample to overflow from the pipette before an aliquot is removed.

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13. Follow the same procedure for the standard and unknown. It is important that each tube be placed in the water bath as soon as the sulfuric acid is added and mixed. To insure that each tube receives the same incubation period, set up a definite timing schedule for each tube.

14. As each tube completes its incubation period, remove it from the water and allow to cool AT LEAST 10 MINUTES.

15. To remove all bubbles, centrifuge the tubes for 30 seconds.

16. Set the wavelength selector of the Spectro-Colorimeter at 550 mμ.

17. Rinse the cuvette of the Spectro-Colorimeter with Precipitating Reagent. (Always rinse the cuvette with the Precipitating Reagent prior to the first sample. Furthermore, because it is difficult to empty the mixture completely from the cuvette, it may be necessary to rinse the cuvette between samples. Be sure to flush the cuvette thoroughly.)

18. Transfer the blank to the cuvette and set on zero absorbance.

19. When inserting the samples into the cuvette, make sure that there is no air space at the tip of the colorimeter pipette. (Because the solution is quite viscous, any air bubbles trapped in the cuvette will be difficult to remove.)

20. Read the absorbance of the unknown and standard at a wavelength of 550 mμ against the blank set at zero absorbance.

NOTES

PATIENTS ON BROMIDE THERAPY: A distinct yellow color in the supernatant (in contrast to the usual straw color) indicates the presence of bromides. Bromides are known to interfere with the cholesterol reaction but can be eliminated by a batchwise use of an ion exchange resin.* However, it may be simpler to wait until bromides have been discontinued before running a cholesterol determination.

*Chiamori, N. and Henry, R. Am. J. Clin. Path. *31*, 305 (1959)

CALCULATION

$$\frac{\text{O.D. Unknown}}{\text{O.D. Standard}} \times 200 = \text{mg. cholesterol/100 ml.}$$

USER'S OWN CHEMICALS

Ferric Chloride Stock Reagent

Dissolve 850 mg of $FeCl_3 \cdot 6H_2O$ in glacial acetic acid and make up to 100 ml in a volumetric flask. The solution is stable when solidified at reduced temperature.

Precipitating Reagent

Dilute 10 ml of the stock ferric chloride solution to 100 ml with glacial acetic acid. For increased stability, store in a refrigerator.

Diluting Reagent

Dilute 8.5 ml of the ferric chloride stock reagent to 100 ml with glacial acetic acid. The solution is stable indefinitely.

Cholesterol Working Standard

Dissolve 200 mg of Cholesterol in glacial acetic acid and make up to 100 ml in a volumetric flask.

Concentrated Sulfuric Acid

7/62 4M NP

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UREA NITROGEN

Ultramicro Adaptation of the Method of Fawcett and Scott

This technical bulletin gives an alternate procedure for determining urea nitrogen in blood serum with the Beckman Ultramicro Analytical System. Unlike the ten "packaged" determinations, however, prepared reagents (Chempak) and a kit of pipettes (Micropak) are not available from Beckman for this determination.

Reagents may be ordered from your laboratory supply dealer; instructions for making them up are given below under "Reagents." The required micropipettes with their Beckman part numbers are listed under "Apparatus," and may be ordered from your dealer or directly from the Spinco Division of Beckman Instruments, Inc., Palo Alto, California.

PRINCIPLE

Serum is incubated with urease in order to hydrolyze the urea content. The ammonia that forms is treated with phenol and alkaline hypochlorite reagents in the presence of a sodium nitroprusside catalyst. Color development takes place within 15 minutes at 37°C or 30 minutes at room temperature. The absorbance of the blue color is a linear function of urea nitrogen concentration.

APPARATUS

		Part Numbers
400 μl micro test tubes		314326
Micropipettes:	5 μl (1 required)	315151
	10 μl (1 required)	315152
	20 μl (1 required)	315153
	80 μl (1 required)	315158
	150 μl (1 required)	315160
	180 μl (1 required)	315161
Beckman Model 151 Spectro-Colorimeter		314100
Beckman Model 154 Micromixer		315300
Constant Temperature Water Bath		

REAGENTS

Reagents are available from the Hyland Laboratories, 4501 Colorado Blvd., Los Angeles 39, Calif.

Buffered Urease

Dissolve 200 mg disodium ethylenediamine tetra-acetate (Fisher Certified) and 6 mg urease (Sigma Chemical Company, Type V) with 20 ml of ammonia-free distilled water. This preparation is stable for one month when stored at 2° to 10°C.

Phenol Color Reagent

Dissolve 5.0 gm of phenol and 25 mg of sodium nitroprusside in water and dilute to 500 ml with ammonia-free distilled water. This solution is stable for one month when stored at 2° to 10°C.

Alkaline Hypochlorite Reagent

Dissolve 2.5 gm of sodium hydroxide and 0.21 gm of sodium hypochlorite in water and dilute to 500 ml with ammonia-free distilled water. This solution is stable for approximately 6 months when stored at 2° to 10°C.

Nitrogen Stock Standard

Prepare a solution equivalent to 300 mg% urea nitrogen by dissolving 1.412 gm of ammonium sulfate (analytical grade) in 100 ml of ammonia-free distilled water.

Nitrogen Working Standard, 30 mg%

Dilute 20 μl of stock standard with 180 μl of ammonia-free distilled water in a 400 μl micro test tube. Mix thoroughly. Prepare fresh.

PROCEDURE

The chart at the end of this section summarizes the step-by-step directions.

1. Use 400 µl micro test tubes for Blank, Standard, and Unknown. Pipette 80 µl buffered urease into each tube. *For convenience, a series of micro test tubes containing 80 µl of buffered urease may be prepared in advance, capped, and refrigerated. (Do not freeze.)*
2. Into the Blank tube, pipette 5µl of ammonia-free distilled water.
3. Into the Standard tube, pipette 5 µl of working standard.
4. Into the Unknown tube, pipette 5 µl of serum.
5. Place all tubes in a 37°C water bath for 15 minutes.
6. After 15 minutes, transfer 10 µl aliquot of Blank, Standard and Unknown to clean micro test tubes designated Blank, Standard, and Unknown respectively.
7. Into the new Blank tube, pipette 150 µl of phenol color reagent and mix completely with the Micromixer. Then add 150 µl of alkaline hypochlorite and mix completely. *Optical density decreases by 1.5% for every minute interval between the addition of the color reagent and the hypochlorite.*
8. Follow the same procedure for the new Standard and Unknown, mixing completely after adding each reagent.
9. For maximum color development, place the tubes in the 37°C water bath for 15 minutes. Alternatively, allow the tubes to stand for 5 minutes at 50° to 55°C, or for 30 minutes at room temperature. The final color is stable for at least 24 hours.
10. Set the wavelength selector of the Spectro-Colorimeter at 630 mµ. Read the absorbance of Blank, Standard, and Unknown against water set at zero.
11. If the absorbance of the Unknown is above 0.7, repeat the color development (steps 7, 8, and 9) using 5 µl aliquots of Blank, Standard, and Unknown. Read absorbances at a wavelength setting of 630 mµ against water set at zero. Values up to 125 mg% may be determined by this method.
12. For concentrations above 125 mg%, repeat the determination using the 300 mg% stock standard undiluted. Read the absorbances at a wavelength of 460 mµ against water set at zero. Values up to 1000 mg% may be determined by this method.

SUMMARIZED PROCEDURE

	Blank	Standard	Unknown
Buffered Urease	80 µl	80 µl	80 µl
Ammonia-free Distilled Water	5 µl		
Working Standard, 30mg%		5 µl	
Serum			5 µl
Incubate tubes in 37°C water bath for 15 minutes. Transfer aliquot from Blank, Standard, and Unknown tubes to clean tubes.			
Aliquot	10 µl	10 µl	10 µl
Phenol Color Reagent Mix each tube completely after pipetting.	150 µl	150 µl	150 µl
Alkaline Hypochlorite Mix each tube completely after pipetting.	150 µl	150 µl	150 µl

CALCULATIONS

$$\frac{\text{Unknown - Blank}}{\text{Standard - Blank}} \times 30 \text{ (or 300)}$$

= mg Urea Nitrogen/100 ml

NOTES

1. Direct sunlight may decrease optical density by 10%; filament and strip lighting, however, have no effect.
2. If the absorbance reading of the blank is greater than 0.06 when read against water, it is an indication that ammonia contamination has occurred. Use new reagents and repeat the determination.
3. A highly turbid serum sample may cause cloudiness in the final colored solution. To correct turbidity, prepare a serum blank, but add the phenol color reagent to the urease before adding the serum. To determine urea nitrogen concentration in this case, subtract the O.D. reading for *this* blank from the O.D. of the Unknown.
4. This procedure may also be used for determining the ammonia nitrogen content of urine. When measuring ammonia nitrogen, follow the procedure given above but substitute ammonia-free distilled water for the buffered urease and omit the first incubation.

REFERENCES

Fawcett, J. K., Scott, J. E. *J. Clin. Path.* ***13,*** 156 (1960)

Searcy, R. L., Gough, G. S., Korotzer, J. L., Bergquist, L. M. *Amer. J. Med. Technol.* ***27,*** 255 (1961)

Chaney, A. L., Marbach, E. P. *Clin. Chem.* ***8,*** 130 (1962)

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ALKALINE PHOSPHATASE

Ultramicro Adaptation of the Method
of Bessey, Lowry, and Brock

This technical bulletin gives a recommended procedure for determining alkaline phosphatase in blood serum with the Beckman Ultramicro Analytical System. Unlike the ten "packaged" determinations, however, prepared reagents (Chempak) and a kit of pipettes (Micropak) are not available from Beckman for this determination.

Reagents may be ordered from your laboratory supply dealer; instructions for making them up are given below under "Reagents." The required micropipettes with their Beckman part numbers are listed under "Apparatus," and may be ordered from your dealer or directly from the Spinco Division of Beckman Instruments, Inc., Palo Alto, California.

PRINCIPLE

Serum is incubated with buffered substrate p-nitrophenyl phosphate. Enzymatic hydrolysis liberates p-nitrophenol, a yellow salt that indicates the degree to which phosphates are split from the substrate.

APPARATUS

	Part Numbers
400 μl micro test tubes	314326
Micropipettes: 5 μl (2 required)	315151
50 μl (1 required)	315156
100 μl (1 required)	315159
200 μl (1 required)	315162
Beckman Model 151 Spectro-Colorimeter	314100
Beckman Model 152 Microfuge	314300
Constant Temperature Water Bath	

REAGENTS

Reagents are available from the Sigma Chemical Company, 3500 De Kalb St., St. Louis 18, Missouri.

Alkaline Buffer Solution

Use Sigma Stock No. 104-5. The solution is stable for 6 to 12 months when refrigerated.

p-Nitrophenol Standard Solution

Use Sigma Stock No. 104-1. The solution is stable for one year when refrigerated and kept away from light. Dilute the solution 1 to 2 for the working standard.

Phosphatase Substrate

Use Sigma Stock No. 104, 40 mg preweighed capsules. The capsules are stable for one year when frozen.

Hydrochloric Acid, concentrated

Sodium Hydroxide, 0.027N

Dilute 2.7 ml of 1N sodium hydroxide to 100 ml with distilled water.

Alkaline Buffered Substrate

Use 400 μl micro test tubes for storing alkaline buffered substrate. Dissolve 40 mg of the substrate in 10 ml distilled water, and then add 10 ml alkaline buffer solution. Mix the solution and, as soon as possible, transfer 100 μl to each micro test tube. Cap the tubes and store them in an upright position in the freezing compartment of a refrigerator. The solution is stable for 4 to 6 weeks when frozen.

PROCEDURE

The chart at the end of this section summarizes the step-by-step directions.

1. Place three 400 μl micro test tubes, each containing 100 μl alkaline buffered substrate, in a 37°C constant temperature water bath to equilibrate. Use one test tube for Blank, one for Standard, and one for Unknown.
2. Into the Blank tube, pipette 5 μl of distilled water. *As soon as the water is added to the Blank tube, begin timing the 30 minute incubation period. Prepare Standard and Unknown tubes as directed in steps 3 and 4 at 30 second intervals (or any convenient and consistent time interval). Be sure to incubate each tube for 30 minutes.* Cap each tube, mix thoroughly and briefly with the Micromixer, and return the tubes to the water bath.
3. Into the Standard tube, pipette 5 μl working standard, 5 mM/l. Cap the tube, mix, and return it to the water bath.
4. Into the Unknown tube, pipette 5 μl of serum. Cap the tube, mix, and return it to the water bath. *When determining more than one Unknown, add the sera to the tubes at 30 second intervals. Return each tube to the water bath immediately after mixing.*
5. After each tube has been incubated for 30 minutes, remove it from the water bath and add 200 μl sodium hydroxide to stop the reaction. Mix completely.
6. Set the Spectro-Colorimeter wavelength selector at 410 mμ. Set the Spectro-Colorimeter at zero absorbance against water.
7. Transfer Blank to the cuvette and read the alkaline absorbance. Then pipette 5 μl concentrated hydrochloric acid into the cuvette, mix slightly with the colorimeter pipette, and read the acid absorbance. Empty the cuvette and rinse it thoroughly with distilled water.
8. Follow the same procedure for Standard and Unknown.

SUMMARIZED PROCEDURE

	Blank	Standard	Unknown
Alkaline Buffered Substrate	100 μl	100 μl	100 μl
Distilled Water	5 μl		
Begin timing 30 minute incubation period after adding distilled water. Mix and return to water bath.			
Add working standard and serum at 30 second intervals. Mix and incubate 30 minutes.			
Working Standard		5 μl	
Serum			5 μl
Sodium Hydroxide	200 μl	200 μl	200 μl
Add HCl to cuvette and stir slightly with colorimeter pipette.			
Concentrated HCl	5 μl	5 μl	5 μl

CALCULATIONS

$$\frac{\text{O.D.}_{\text{Unknown (A - a)}^*} - \text{O.D.}_{\text{Blank (A - a)}}}{\text{O.D.}_{\text{Standard (A - a)}} - \text{O.D.}_{\text{Blank (A - a)}}} \times 10 = \text{B.L.U. (Bessey Lowry Units)}$$

* (A − a) = (Alkaline minus Acid)

NOTES

1. The alkaline buffered substrate is prepared in bulk. For convenience, it is dispensed into micro test tubes at the time of preparation and then stored in the freezing compartment of a refrigerator. Even under these conditions, the blank value rises slowly. For this reason, a reagent blank is always run and optical density is subtracted in addition to the serum blank. The prepared substrate has been stored for up to six months without a loss of accuracy.
2. The Standard is processed simultaneously with the Unknown so that the same pipettes are used for both, thus any variation in calibration is nullified.
3. The final color is stable.
4. The above procedure allows for readings of up to 10 B.L.U. It is possible to obtain readings as high as 1.0 absorbance or about 12 B.L.U. by reading the percent transmission scale on the Spectro-Colorimeter and then converting the values to optical density before calculations. If the analysis is more than 12 B.L.U., repeat the determination on a diluted sample.

REFERENCES:

Bessey, O. A., Lowry, O. H., Brock, M. J. *J. Biol. Chem.* ***164***, 321 (1946).

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PHOSPHORUS

Ultramicro Adaptation of the Method of Baginski and Zak

This technical bulletin gives an alternate procedure for determining phosphorus in blood serum with the Beckman Ultramicro Analytical System. Unlike the ten "packaged" determinations, however, prepared reagents (Chempak) and a kit of pipettes (Micropak) are not available from Beckman for this determination.

Reagents may be ordered from your laboratory supply dealer; instructions for making them up are given below under "Reagents." The required micropipettes with their Beckman part numbers are listed under "Apparatus," and may be ordered from your dealer or directly from the Spinco Division of Beckman Instruments, Inc., Palo Alto, California.

PRINCIPLE

The proteins in serum are precipitated with trichloroacetic acid. The protein-free filtrate is treated with ammonium molybdate to form phosphomolybdic acid which is reduced by ascorbic acid. The molybdenum blue complex formed is stabilized by citrate-arsenite reagent and color development is complete after 15 minutes. The stable blue color is proportional to the phosphate concentration.

APPARATUS

	Part Numbers
400 μl micro test tubes	314326
Micropipettes: 20 μl (2 required)	315153
50 μl (1 required)	315156
100 μl (2 required)	315159
180 μl (1 required)	315161
200 μl (1 required)	315162
Beckman Model 151 Spectro-Colorimeter	314100
Beckman Model 152 Microfuge	314300
Beckman Model 154 Micromixer	315300

REAGENTS

The user's own chemicals should be prepared according to the instructions below.

Stock Phosphorus Standard, 40 mg/100 ml

Dissolve 0.175 gm potassium dihydrogen phosphate (KH_2PO_4 that has been dried overnight at 80°C and then cooled in a dessicator) in 100 ml of distilled water. Keep under toluene.

Working Phosphorus Standard, 4 mg/100 ml

Dilute 20 μl stock standard with 180 μl distilled water in a 400 μl micro test tube. Mix thoroughly. Prepare fresh daily.

Ammonium Molybdate Solution, 1%

Dissolve 1 gm of ammonium molybdate tetrahydrate in distilled water in a 100 ml volumetric flask. Dilute to volume with distilled water.

Arsenite-Citrate Reagent

Dissolve 2 gm each of sodium citrate and sodium arsenite in about 80 ml of distilled water in a 100 ml volumetric flask. Add 2 ml of glacial acetic acid and dilute to volume with distilled water.

Ascorbic Acid Solution, 10%

Dissolve 10 gm ascorbic acid, Merck U.S.P. medium crystals, in distilled water in a 100 ml volumetric flask. Dilute to volume with distilled water.

Trichloroacetic Acid, 10%

Dissolve 10 gm trichloroacetic acid in distilled water in a 100 ml volumetric flask. Dilute to volume with distilled water.

PROCEDURE

The chart at the end of this section summarizes the step-by-step directions.

1. Use 400 μl test tubes for Blank, Standard, and Unknown.
2. Into the Blank tube, pipette:
   20 μl distilled water
   200 μl trichloroacetic acid
3. Into the Standard tube, pipette:
   20 μl working standard
   200 μl trichloroacetic acid
4. Into the Unknown tube, pipette:
   20 μl serum
   200 μl trichloroacetic acid
5. Cap the tubes and mix with the Micro-mixer. Allow tubes to stand for three minutes.
6. Centrifuge tubes for 30 seconds with the Microfuge.
7. Transfer 100 μl aliquot of Blank, Standard, and Unknown to clean micro test tubes designated Blank, Standard, and Unknown respectively.
8. Into the new Blank, Standard, and Unknown tubes, pipette in the following order:
   20 μl ascorbic acid
   50 μl ammonium molybdate
   100 μl citrate-arsenite reagent

   NOTE: Mix after each pipetting.
9. Allow the tubes to stand for 15 minutes for color development.
10. Set the wavelength selector of the Spectro-Colorimeter at 650 mμ. Read absorbance of Blank, Standard, and Unknown against water set at zero.

SUMMARIZED PROCEDURE

	Blank	Standard	Unknown
Distilled Water	20 μl		
Standard		20 μl	
Serum			20 μl
Trichloroacetic Acid, 10%	200 μl	200 μl	200 μl
Mix, wait three minutes, then centrifuge tubes for 30 seconds. Transfer aliquot from Blank, Standard, and Unknown tubes to clean tubes.			
Aliquot	100 μl	100 μl	100 μl
Add the following reagents in the order given, i.e., acid, then molybdate, then citrate-arsenite. Mix after each pipetting.			
Ascorbic Acid	20 μl	20 μl	20 μl
Ammonium Molybdate	50 μl	50 μl	50 μl
Citrate-Arsenite	100 μl	100 μl	100 μl

CALCULATIONS

$$\frac{\text{Unknown - Blank}}{\text{Standard - Blank}} \times 4 = \text{mg Phosphate/100 ml}$$

NOTES

1. It is important to add the reagents in the order given. To stabilize the color development, add the citrate-arsenite reagent immediately after adding the ammonium molybdate.
2. The ascorbic acid solution is stable for one week at room temperature. Prepare working phosphorus standard daily and ascorbic acid solution weekly. All other reagents are stable.

REFERENCES

Baginski, E., and Zak, B. *Clin. Chim. Acta* ***5,*** 834 (1960)

Method of Analysis with the Beckman Model 150 Ultramicro Analytical System

GENERAL CONSIDERATIONS

CHOICE OF METHOD

The particular method chosen to carry out each of the analyses offered has been selected because, generally speaking, it (1) lends itself to ultramicro adaptation, (2) has proven to be a popular procedure in the field, and (3) should enhance ultramicro development.

GENERAL PRECAUTIONS

To obtain accurate results, the user should observe the following precautions when he carries out procedures:

Clean Laboratory

The laboratory in which work is done must be clean. Impurities in the air, such as dust and chemicals, may cause major errors in work. Air-conditioning should incorporate a dust filter.

Pure Water

Distilled water of high purity must be used for all solutions and for washing, unless otherwise stated.

Reagents

Variations in climate make it difficult to dictate shelf life for even the most stable chemical preparation. Reagents must be discarded when they show signs of deterioration.

A reagent that is recommended to be kept under refrigeration must be put back in the refrigerator immediately after use.

A user who mixes his own reagents should use chemicals having a high degree of purity.

HOW TO TAKE CAPILLARY BLOOD

Serum Method

1. Seal a glass capillary tube having the following dimensions: 75 mm length, 1.5 mm inner diameter, and 3 mm outer diameter. Place the sealed capillary tube into a microtube.
2. Using an antiseptic, clean and dry the side of a finger, toe, or heel.
3. Make a sufficiently deep prick with a sterile, disposable lancet so that blood flows freely when gentle pressure is applied. Do not squeeze the puncture site after the blood flow is started, since tissue fluids tend to reduce the concentration of blood constituents. If the blood begins to clot, gentle massage with an alcohol-soaked sponge should restore the flow.
4. Holding the microtube vertically, allow blood to run down the outside of the capillary tube until the microtube is full.
5. Remove the glass capillary tube. The specimen is now ready for centrifugation.

Plasma Method

Use the same technique as above, substituting an unsealed, heparinized capillary tube of the same dimensions. Let the blood flow down the inside of the capillary tube to the bottom of the microtube. If the blood does not flow freely, a flick of the wrist will help drive it to the bottom of the tube. Be sure to select an anti-coagulant that will not contaminate the plasma when the plasma is analyzed for other constituents.

Use serum for analysis whenever possible.

Amount of Specimen

If the skin has been punctured correctly, the microtube can be completely filled. This provides enough serum or plasma for several determinations.

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7/62 2.5M NP

Technical Bulletin No. 6072C

Method of Analysis with the Beckman Model 150 Ultramicro Analytical System

CHLORIDE

Ultramicro Adaptation of the Method of Schales and Schales

PRINCIPLE

Serum is titrated with an acid solution of mercuric nitrate in the presence of s-diphenyl carbazone indicator. The chloride ions present react with the mercuric ions to form soluble but undissociated mercuric chloride. Excess mercuric ions at the end-point of the titration form a blue-violet complex salt with s-diphenylcarbazone.

PROCEDURE

1. Using the micro titration cups, prepare blank, working standard and unknown.

	Blank	Standard	Unknown
Distilled Water	10μl.*		
Standard		10μl.	
Serum			10μl.
Nitric Acid	80μl.	80μl.	80μl.
Indicator	80μl.	80μl.	80μl.
	Mix	Mix	Mix

*The same pipette is used for blank, standard and serum. Rinsing is done by allowing one drop of sample to overflow from the pipette before an aliquot is removed.

2. In the above order, mix and titrate with mercuric nitrate until the indicator produces a faint blue-violet color.

CALCULATIONS

Milliequivalents of Chloride Per Liter

Calculate as follows to express results in milliequivalents of chloride per liter (*mEq. Chloride/L.*). Subtract the Microtitrator dial reading obtained with blank from the readings obtained with unknown and standard; then multiply the ratio of net unknown to net standard by 100 to obtain the milliequivalents of chloride per liter. Use this equation:

$$\frac{\text{Unknown}-\text{Blank}}{\text{Standard}-\text{Blank}} \times 100 = mEq.\ Chloride/L.$$

Milligrams of Chloride Per 100 Milliliters

Calculate as follows to express results in milligrams of chloride per 100 milliliters (*mg. Chloride/100 ml.*). Multiply the milliequivalents of chloride per liter (*mEq. Chloride/L.*), by 5.85 to obtain a result expressed in milligrams of chloride per 100 milliliters. Use this equation:

$$mEq.\ Chloride/L. \times 5.85 = mg.\ Chloride/100\ ml.$$

NOTES

1. Schales and Schales were first to use s-diphenylcarbazone as an indicator. This is a procedure analogous to that employed by Volhard.
2. The indicator is added during the mixing of the solution, just before each sample is titrated. The micro titration cup is already in position and the stirrer has been started.

REAGENTS

Either prepare reagents as described below; or, alternatively, use the Chloride Chempak instructions.

User's Own Chemicals

Working Chloride Standard, 100 mEq./L:

Dissolve 0.584 gm. previously dried reagent grade sodium chloride in distilled water and dilute to 100 ml. Store in 30 ml. polyethylene bottles.

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Mercuric Nitrate Reagent, 0.1N:

Dissolve 1.6 gm. reagent grade mercuric nitrate in distilled water containing 1 ml. concentrated nitric acid. Dilute to 100 ml. distilled water and mix. Store in 30 ml. polyethylene bottles.

Nitric Acid, 0.03N:

Dilute 0.2 ml. concentrated nitric acid to 100 ml. with distilled water. Store in 30 ml. polyethylene bottles.

Indicator Solution:

Dissolve 100 mg. of s-diphenylcarbazone in 95% ethyl alcohol and dilute to 100 ml. Store in brown glass 30 ml. bottles. Keep in refrigerator when not in use. The solution should be orange-red. If it turns yellow, it should be discarded.

Chloride Chempak: How to Mix Chemicals

Indicator:

1. Carefully remove one of the plugs from a S-DIPHENYLCARBAZONE container.
2. Empty the contents into the 30 ml. bottle labeled ETHYL ALCOHOL.
3. Remove the remaining plug and drop both pieces into this same bottle, together with the plug removed first.
4. Cap this bottle. Mix by shaking by hand.
5. Pour the colored solution into the 30 ml. bottle labeled INDICATOR.
6. Attach the 80μl. pipette to the INDICATOR bottle.
7. Keep in refrigerator when not in use. The solution should be orange-red. If it turns yellow, replace it.

REFERENCES

1. *Schales, O., and Schales, S. S.: J. Biol. Chem. 140, 879, 1941*
2. *Volhard, J. J.: Prakt. Chem., 9, 217, 1874*
3. *Volhard, J.: Z. Anal. Chem., 17, 482, 1878*

Technical Bulletin No. 6073D

Method of Analysis with the Beckman Model 150 Ultramicro Analytical System

GLUCOSE

Ultramicro Adaptation of the Method of Keston and Teller

PRINCIPLE

"Glucostat" is a coupled enzyme system, utilizing glucose-oxidase-peroxidase, and a chromogen o-dianisidine, which oxidizes true glucose to gluconic acid and hydrogen peroxide. The oxygen produced by the reaction of peroxidase on hydrogen peroxide, reacts with the chromogenic acceptor to form a stable color complex. The intensity of this color is proportional to the glucose concentration.

The "glucostat" does not determine other hexoses, pentoses, or reducing substances.

SCREENING PROCEDURE

1. Using micro test tubes, prepare blanks, working standard and unknown.

	Blank for Standard	Standard	Blank for Unknown	Unknown
Glucostat	250 µl.	250 µl.	250 µl.	250 µl.
Hydrochloric Acid	10 µl.		10 µl.	

The same pipette is used for blank, standard and unknown. Rinsing is done by allowing the first drop of each aliquot to overflow.

2. Use sample pipette to add 5 µl. water to blank-for-standard tube and 5 µl. serum to blank-for-unknown tube. Mix.
3. Start stopwatch and immediately add 5 µl. working standard to standard tube and mix.
4. After 30 seconds, or a convenient time interval, add 5 µl. serum to unknown tube and mix.
5. Stop reaction in the standard tube 10 minutes after the working standard was added to it by adding 10 µl. hydrochloric acid and mixing. Likewise, stop reaction in the unknown tube 10 minutes after the serum was added to it by adding 10 µl. hydrochloric acid and mixing.
6. Read absorbances at wavelength of 410 mµ. on the Beckman/Spinco Model 151 Spectro-Colorimeter. The standard is read against the blank-for-standard set at zero absorbance, and the unknown is read against the blank-for-unknown set at zero absorbance.
7. When more than one serum is run at a time, a blank-for-unknown is required for each, and the same 10-minute time interval between the adding of serum to Glucostat and the stopping of the reaction with hydrochloric acid must be maintained for each.

ALTERNATIVE PROCEDURE

1. If it is felt that deproteinization is required as, for example, in the case of hemolyzed or icteric serum or plasma, a 1:20 filtrate is prepared. This can be done by adding 4 parts of 5% $ZnSO_4 \cdot 7\,H_2O$ and 15 parts of 0.08 N NaOH to one part of distilled water for the blank, to one part of the working standard, and to one part of each serum or plasma being tested.
2. Micropipettes from the Sparepak can be conveniently used for this purpose as follows:

	Blank	Standard	Unknown
Distilled Water	10 µl.		
Working Standard		10 µl.	
Serum			10 µl.
Sodium hydroxide	150 µl.	150 µl	150 µl.
Zinc sulfate	40 µl.	40 µl.	40 µl.

3. Mix and centrifuge one minute. Pipette 100 µl. aliquots of supernatant into clean micro test tubes.

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	Blank	Standard	Unknown
Transfer pipette	100 µl.	100 µl.	100 µl.
Hydrochloric Acid	10 µl.		
Glucostat	250 µl.		
	Mix		

4. Start stopwatch and immediately add 250µl. Glucostat to standard tube.
5. After 30 seconds, or a convenient time interval, add 250µl. Glucostat to unknown tube.
6. Stop reaction in standard tube 10 minutes after adding the Glucostat by adding 10µl. hydrochloric acid and mixing. Likewise, stop reaction in the unknown tube 10 minutes after adding the Glucostat to that tube by adding 10µl. hydrochloric acid and mixing.
7. Read absorbance of standard and unknown at wavelength of 410mµ. on the Beckman/Spinco Model 151 Spectro-Colorimeter against the blank set at zero absorbance.
8. When more than one serum is run at a time the same 10-minute interval between the adding of Glucostat to the unknown tube and the stopping of the reaction with hydrochloric acid must be maintained for each.

CALCULATIONS

Multiply by 100 the ratio of the Spectro-Colorimeter absorbance reading obtained with unknown to the reading obtained with standard, to obtain a result expressed in milligrams of glucose per 100 milliliters. Use this equation:

$$\frac{\text{Unknown}}{\text{Standard}} \times 100 = \text{mg. } \textit{Glucose}, \text{ 100 ml.}$$

NOTES

1. Hemoglobin and other unknown blood substances interfere with the reaction; thus the alternative procedure, which includes deproteinization, is recommended for reliable results. Bilirubin, thymol, ascorbic acid, uric acid, and catechols compete as hydrogen donors and are potential inhibitors of color development.
2. Serum should be separated from the cells as soon as possible.

REAGENTS

Either prepare reagents as described below; or, alternatively, use the Glucose Chempak instructions.

User's Own Chemicals

Stock Glucose Standard, 1000 mg./100 ml.:
Dissolve 1.000 gm. dry reagent grade glucose (dextrose) in distilled water and dilute to 100 ml. Add 0.1 gm. benzoic acid and mix.

Working Glucose Standard, 100 mg./100 ml.:
Dilute 20µl. stock standard with 180 µl. distilled water in a 400µl. micro test tube. Mix thoroughly. Prepare fresh daily.

Hydrochloric Acid, 5N:
Dilute 42 ml. concentrated HCl reagent grade to 100 ml. with distilled water. Store in 30 ml. polyethylene bottles.

Sodium Chloride:
Dissolve 0.85 gm. of reagent grade NaCl in distilled water and dilute to 100 ml. Store in 30 ml. polyethylene bottles.

Glucostat Reagent:
This reagent is prepared from constituents for enzymatic glucose reagent obtained from Worthington Biochemical Corporation, Freehold, New Jersey.
Prepare the Glucostat Reagent in the following manner:
Pour 50 ml. of NaCl solution into a 100 ml. graduated cylinder. Now add 1 ml. of METHANOL to the small vial labeled CHROMOGEN and pour this mixture into the graduated cylinder of saline solution. Do not rinse the CHROMOGEN vial, otherwise turbidity will result.
Dissolve the contents of the large vial with the same sodium chloride solution and add this to the same graduated cylinder. Carefully rinse twice and complete the volume to *81 ml.* with sodium chloride solution.
Pour this solution into a brown polyethylene bottle and store in a refrigerator at about 4° C. This solution can be stored up to one week if left in a refrigerator.
Before the determination, pour out the volume needed into a small container and filter through a fast filter paper.

Zinc Sulfate, 5% $ZnSO_4 \cdot 7H_2O$:
Dissolve 5 grams of reagent grade $ZnSO_4 \cdot 7H_2O$ in distilled water and dilute to 100 ml.

Sodium Hydroxide, 0.08 N:
Dissolve 3.2 grams of reagent grade NaOH in distilled water and dilute to 1 liter. 15 parts of the 0.08 N NaOH should be equivalent to 4 parts of the 5% $ZnSO_4 \cdot 7\ H_2O$. Titrate one against the other, using phenolphthalein as indicator. Dilute the stronger to match the weaker

How to Mix Chemicals Glucose Chempak:

Glucostat Reagent:
1. Dilute 3.0 ml. of SODIUM CHLORIDE solution to 100 ml. with distilled water. Pour 50 ml. of this solution into a 100 ml. graduated cylinder. Now add 1 ml. of METHANOL to the small vial labeled CHROMO

GEN and pour this mixture into the graduated cylinder of saline solution. Do not rinse the CHROMOGEN vial, as this will cause turbidity.

2 Dissolve the contents of the large vial, GLUCOSTAT, with the same saline solution and add this to the same graduated cylinder. Carefully rinse twice and complete the volume to *81 ml.* with saline solution. Mix by shaking by hand.

3. Pour this solution into a brown polyethylene bottle and store in a refrigerator at about 4° C. This solution can be stored up to one week if left in a refrigerator.

4 Before the determination, pour out the volume needed into a small container and filter through a fast filter paper into the pipette bottle labeled GLUCOSTAT. Attach the 250 µl. pipette to this bottle.

Working Standard:

1. Using the STOCK TRANSFER pipette, transfer 20µl. of STOCK STANDARD to a 400µl. micro test tube.
2. Add 180µl. of water to this tube from the DISTILLED water pipette.
3. Mix: either flick wrist, then tap bottom of tube; or, alternatively, use Micromixer.
4. Prepare fresh daily.

REFERENCES

1. *Keston, A. S.: Abstracts of Papers, 129th Meeting, ACS, Page 31c, April, 1956*
2. *Teller, J. D.: Abstracts of Papers, 130th Meeting, ACS, Page 69c, Sept. 1956*
3. *Detailed instructions by Worthington Biochemical Corporation, Freehold, New Jersey; modified by Sanz.*

7/62 3M NP

Technical Bulletin No. 6074D

TOTAL PROTEIN

Method of Analysis with the Beckman Model 150 Ultramicro Analytical System

Total, Albumin, Albumin/Globulin Ratio: Ultramicro Adaptation of the Method of (1) Kingsley (2) Gornall, Bradawill and David

PRINCIPLE

A modified biuret reagent is used to produce a blue-purple copper complex when added to dilute serum or its ether extract. The color produced, which is proportional to the protein concentration, is then compared with a standard protein solution which has been treated similarly and the total protein and albumin values are determined. The difference between these values is essentially globulin.

PROCEDURE

1. Using 400μl. micro test tubes, prepare blanks, working standard, unknown total and albumin. (*Use the 5 μl. sample pipette twice for albumin.*)

	Blank	Standard	Total	Albumin
Distilled Water	5μl.*			
Standard		5μl.		
Serum			5μl.	10μl.
Sodium Sulfite				100μl.
				Mix gently
Ether				100μl.

*The same pipette is used for blank and standard. Rinsing is done by allowing one drop of sample to overflow from the pipette before an aliquot is removed.

2. Place the stopper in the mouth of the albumin tube and shake for 20 seconds. *(2 shakes per second.)* Shake carefully; ether leaks readily from the capped tube.
3. Centrifuge albumin tube for 30 seconds.
4. With a sharp blade, slice through the micro test tube just below the globulin button; discard the upper part of the tube, and pipette 60μl. of clear solution from the lower end of the tube.
5. Transfer this aliquot to a clean micro test tube.
6. Add biuret as follows:

	Blank	Standard	Total	Albumin
Biuret Reagent	200μl.	200μl.	200μl.	200μl.
	Mix	Mix	Mix	Mix
Sodium Sulfite	50μl.	50μl.	50μl.	
	Mix	Mix	Mix	

7. Thirty minutes after the addition of biuret, read the absorbance of unknown and standard at wavelength of 540 mμ. on the Beckman/Spinco Model 151 Spectro-Colorimeter against the blank set at zero absorbance.

CALCULATIONS

To convert protein nitrogen (mg per ml) to protein (gm per 100 ml), multiply the value on the ampoule by 0.625.

Total Protein

Multiply the ratio of the Spectro-Colorimeter absorbance reading for the unknown total to the reading obtained with standard, by the amount of protein in the standard, to obtain grams of total protein per 100 milliliters. Use this equation:

$$\frac{\text{Unknown total}}{\text{Standard}} \times \text{protein in standard} = \text{gm. total protein/100 ml.}$$

Albumin

Multiply the ratio of the Spectro-Colorimeter absorbance reading for the unknown albumin to the reading obtained with standard, by the amount of albumin in the standard, to obtain grams of albumin per 100 milliliters. Use this equation:

$$\frac{\text{Unknown albumin}}{\text{Standard}} \times \text{protein in standard} \times 0.935 = \text{gm. albumin/100 ml.}$$

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NOTES

1. There must be no hemolysis of cells, and serum should be separated as soon as possible.
2. Too vigorous shaking of the serum-salt mixture with ether should be avoided to prevent denaturation of the albumin. Too little shaking will result in incomplete separation and lead to falsely high albumin values.
3. Sodium sulfite, 28%, should be stored in an incubator at 37° C.

REAGENTS

Either prepare reagents as described below; or, alternatively, use the Protein Chempak instructions.

User's Own Chemicals

Working Protein standard:

Several brands of albumin are available from chemical supply companies. It is important to use the best grade available.

Biuret Reagent (*Gornall*):

Dissolve 0.15 gm. reagent copper sulfate, $CuSO_4 \cdot 5H_2O$, and 0.60 gm. reagent-grade sodium-potassium tartrate, $NaKC_4H_4O_6 \_ 4H_2O$, into 50 ml. distilled water in • 100 ml. volumetric flask. Add, with stirring, 30 ml. 10% (2.5N) carbonate-free sodium hydroxide. Dilute to the mark and mix. Store in brown 30 ml. polyethylene bottles.

Sodium Sulfite, 28%:

Dissolve 28.0 gm. reagent-grade anhydrous sodium sulfite, Na_2SO_3, in distilled water and dilute to 100 ml. Store in 30 ml. polyethylene bottle.

Ether, reagent grade:

Store in tightly-closed 30 ml. glass bottles.

Total Protein Chempak: How to Mix Chemicals

Working Standard:

1. File a scratch mark around the blue ring on the neck of the WORKING STANDARD ampoule.
2. Break off the top. Withdraw the amount of solution required.
3. Mold a piece of Parafilm over the broken edge of the ampoule.
4. Store the ampoule in the refrigerator.

REFERENCES

1. *Kingsley, G. R.: J. Biol. Chem., 131, 197, 1939.*
2. *Gornall, A. G., Bardawill, C. J. and David, M. M.. J. Biol. Chem., 177, 1949*

7/62 2.5M NP

Technical·Bulletin No. 6075E

Method of Analysis with the Beckman Model 150 Ultramicro Analytical System

UREA NITROGEN

Ultramicro Adaptation of the Method of Fearon and Friedman

PRINCIPLE

The urea in a protein-free filtrate is heated with diacetyl monoxime and the color developed by treatment with arsenic-sulfuric acid reagent. By oxidizing the interfering hydroxylamine which forms, the acid reagent eliminates any interference to color development.

PROCEDURE

1. Using 400μl. micro test tubes, prepare blank, working standards, and unknown as follows:

	Blank	$Standard_{20}$	Unknown
Distilled Water	10μl.*		
$Standard_{20}$		10μl.	
Serum			10μl.
Trichloro-acetic Acid	40μl.	40μl.	40μl.

*The same pipette is used for blank, $standard_{20}$ and serum. Rinsing is done by allowing one drop of sample to overflow from the pipette before an aliquot is removed.

2. Mix and centrifuge 30 seconds. Use the 20μl. transfer pipette to transfer an aliquot of each supernatant into a clean 400μl. micro test tube. Treat as follows:

	Blank	$Standard_{20}$	Unknown
Aliquot of Supernatant	20μl.	20μl.	20μl.
Diacetyl Monoxime	20μl.	20μl.	20μl.
	Mix	Mix	Mix
Arsenic Sulfuric Acid	40μl.	40μl.	40μl.
	Mix	Mix	Mix
Distilled Water	180μl.	180μl.	180μl.
	Mix	Mix	Mix

3. Make a boiling water bath by filling a 100 ml. beaker with water to within about ⅜ inch of the top; bring to a slow boil.
4. Hang the tubes in the beaker with the clips resting on the rim. Bring the solution in the tubes to a boil with the tubes uncapped.
5. Quickly remove each tube and place its cap on tightly. Return the tube to the beaker and allow it to boil for 20 minutes. Add boiling water to the beaker as necessary to keep the water level at the top of the tubes.
6. Cool for 3 minutes in a beaker of cool tap water. Invert tubes to collect distillate from tube tops, then set right side up again.
7. Read within 7 minutes.
8. Read absorbances of unknown and standards, within 7 minutes, at wavelength of 475 mμ. on the Beckman/Spinco Model 151 Spectro-Colorimeter against the blank set at zero absorbance.

CALCULATIONS

$$\frac{\text{Optical Density}_{\text{Unknown}}}{\text{Optical Density}_{\text{Standard}}} \times 20 = \text{mg. urea nitrogen/100ml.}$$

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NOTES

1. Diacetyl reacts with urea in strong acid solution to produce a yellow color with maximum absorption at 475 mμ. Citrulline and other substances carrying a urea residue in the molecule also react. The color with citrulline is pink. Interference from these substances in serum is negligible.
2. The final diacetyl color cannot be diluted: repeat determination on diluted filtrate.

REAGENTS

Either prepare reagents as described below; or, alternatively, follow the Urea Nitrogen Chempak instructions.

User's Own Chemicals

Stock Urea Standard, 100 mg./100 ml.:
Dissolve 0.2144 gm. urea (dried in a desiccator) and make up to 100 ml. with distilled water. Store in brown 30 ml. polyethylene bottles to which 1 ml. of toluene has been added. Keep in refrigerator.

Working Urea Standard, 20 mg./100 ml.:
Dilute 20μl. stock standard with 80μl. distilled water in a 400μl. micro test tube. Mix thoroughly. Prepare fresh daily.

Arsenic-Sulfuric Acid:
While carefully stirring and cooling, add 50 ml. concentrated reagent grade sulfuric acid to 50 ml. distilled water in a 500 ml. Erlenmeyer flask. After cooling mixture to about 50°C., add 10 gm. reagent-grade arsenic acid, H_3AsO_4, mix well and allow to cool. Store in 30 ml. polyethylene bottles.

Diacetyl Monoxime, 1%:
Dissolve 1.0 gm. diacetyl monoxime (*Eastman*) in 100 ml. 5% reagent-grade acetic acid. Store in brown 30 ml. polyethylene bottles. Keep in refrigerator when not in use.

Trichloroacetic Acid, 10%:
Dissolve 10 gm. reagent-grade crystalline trichloroacetic acid in distilled water and dilute to 100 ml. Store in 30 ml. polyethylene bottles.

Urea Nitrogen Chempak: How to Mix Chemicals

Stock Standard:
1. Empty the contents of the vial labeled dry UREA into a 1000 ml. volumetric flask.
2. Using distilled water, rinse the remaining contents from the vial into the flask.
3. Dissolve the dry UREA and add sufficient distilled water to make 1000 ml.
4. Mix thoroughly.
5. Cover with layer of toluene and store in refrigerator.

Working Standard(20):
1. Using the STOCK TRANSFER pipette, transfer 20μl. of STOCK STANDARD to a 400μl. micro test tube.
2. Use the 80μl. distilled water pipette to add 80μl. of distilled water to micro test tube. Mix thoroughly.
3. Prepare fresh daily.

Diacetyl Monoxime:
1. Pour contents of ACETIC ACID bottle onto the dry diacetyl monoxime in the 30 ml. bottle labeled DIACETYL MONOXIME.
2. Cap this bottle. Mix by shaking by hand until the solid chemical dissolves.
3. Attach a 20μl. pipette to the DIACETYL MONOXIME bottle.

REFERENCES

Fearon, W. R.: Biochem. J. 33, 902, 1939
Friedman, H. S.: Anal. Chem., 25, 662, 1953

7/62 3.5M NP

Technical Bulletin No. 6076D

Method of Analysis with the Beckman Model 150 Ultramicro Analytical System

BILIRUBIN

Ultramicro Adaptation of the Method of Malloy and Evelyn

PRINCIPLE

Investigations based on van den Bergh and Ehrlich reactions have detected bilirubin in two forms. Bilirubin glucuronide can be measured by a *direct reaction* in an aqueous serum dilution. Alcohol-soluble bilirubin can be determined in a 50% methanolic solution by a *total reaction*, which measures both forms of bilirubin. The addition of diazo reagent to each dilution produces a pink-violet color of azobilirubin proportional to the bilirubin concentration. Spectrophotometric absorbance readings are made at one minute for the direct reaction and at 30 minutes for the total reaction.

APPARATUS

400μl micro test tubes
Bilirubin Micropak
  Sample Pipette: 20μl
  Reagent Pipettes: 30μl, 100μl, 150μl, 250μl
Bilirubin Chempak
Beckman Model 151 Spectro-Colorimeter
Beckman Model 154 Micromixer

REAGENTS: Bilirubin Chempak

Stock Standard

1. Use the point of a knife to pry the lid from the container. Be careful not to lose any of the contents.

2. Drop the container and lid into a dry 125 ml Erlenmeyer flask.

3. Add approximately 70 ml chloroform supplied with the Chempak.

4. Swirl the flask to mix thoroughly. Generally, a solution will be formed within 5 to 10 minutes.

5. Pour solution through a dry funnel into a dry 100 ml glass-stoppered volumetric flask.

6. Wash with several portions of chloroform, adding to the flask. Then dilute to exactly 100 ml at 25°C. Mix by inversion.

7. Dry the brown glass bottles, which contained the chloroform, by inverting for several minutes. Pour the stock standard just prepared, 40 mg/100 ml, into these bottles.

8. Screw the caps on tightly. Store in a refrigerator when not in use.

Working Standard

1. Using the stock transfer pipette, transfer 20μl of stock standard at 25°C to a 400μl micro test tube.

2. Using the chloroform pipette, add 180μl of chloroform at 25°C to the tube. Mix thoroughly with the Micromixer.

3. Prepare fresh working standard just before use. Keep capped and discard the excess.

Diazo Reagent

1. Fill a 50 ml volumetric flask about half full with distilled water. Drop a diazo tablet into the flask.

2. Add 1.5 ml of hydrochloric acid (for diazo).

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3. Dissolve the tablet. Add sufficient water to make 50 ml of solution. Cap the flask and mix by inversion until a solution is formed.

4. Filter the solution and transfer the filtrate into the diazo reagent pipette bottle.

5. Attach 30μl pipette to this bottle.

6. Prepare fresh diazo reagent just before use.

PROCEDURE

1. Prepare fresh working standard and diazo reagent. The working standard is extremely volatile and must be protected from evaporation. It should be prepared immediately before use, capped between aliquots, and discarded after use.

2. Use 400μl micro test tubes for Blank and Standard; Total Blank and Unknown; and Direct Blank and Unknown. The chart at the end of the procedure summarizes the step-by-step directions.

   NOTE: After each pipetting, mix with the Micromixer.

3. Into the Blank Standard tube, pipette:
   250μl of methanol
   30μl of hydrochloric acid
   20μl of standard

4. Into the Standard tube, pipette:
   250μl of methanol
   30μl of diazo reagent
   20μl of standard

5. Into the Total Blank tube, pipette:
   100μl of distilled water
   150μl of methanol
   30μl of hydrochloric acid
   20μl of serum

6. Into the Total Unknown tube, pipette:
   100μl of distilled water
   150μl of methanol
   30μl of diazo reagent
   20μl of serum

   NOTE: Cap the tubes, mix thoroughly, and allow 30 minutes for color development. During this time, proceed with steps 7, 8, 9, and 10.

7. Set the Spectro-Colorimeter at zero absorbance against water. Set the wavelength selector at 540 mμ.

8. Into the Direct Blank tube, pipette:
   250μl of distilled water
   30μl of hydrochloric acid
   20μl of serum

9. *NOTE: Since the direct reading is taken only one minute after the addition of serum, only one direct determination can be done at a time.*

   Into the Direct Unknown tube, pipette:
   250μl of distilled water
   30μl of diazo reagent
   20μl of serum
   CAP TUBE AND MIX IMMEDIATELY.

10. One minute after addition of serum in step 9, read absorbance of Direct and Blank against water.

11. Thirty minutes after preparing Standard, Total, and Blanks, read absorbances against water at 540 mμ. When reading absorbance of alcoholic solutions, use special care to avoid bubbles in the cuvette.

Summarized Procedure

	Standard		Total		Direct	
	Blank	Standard	Blank	Unknown	Blank	Unknown
Distilled Water			100μl	100μl	250μl	250μl
Methanol	250μl	250μl	150μl	150μl		
Hydrochloric Acid	30μl		30μl		30μl	
Diazo Reagent		30μl		30μl		30μl
Standard*	20μl	20μl				
Serum*			20μl	20μl	20μl	**20μl

*The same pipette is used for Blank, Standard, and Unknown. To rinse the pipette, allow one drop of sample to overflow the pipette before removing an aliquot.
**Mix immediately with the Micromixer.

CALCULATIONS

Total Bilirubin

$$\frac{\text{Total Unknown}-\text{Blank}}{\text{Standard}-\text{Blank}} \times 4 = mg\,total\,bilirubin/100\,ml$$

Direct Bilirubin

$$\frac{\text{Direct Unknown}-\text{Blank}}{\text{Standard}-\text{Blank}} \times 4 = mg\,direct\,bilirubin/100\,ml$$

NOTES

1. Bilirubin should be determined within two hours after the blood is drawn. Serum should be clear and not hemolyzed.

2. Malloy and Evelyn were first to find that 50% methanol gave the maximum color for the total reaction.

USER'S OWN CHEMICALS

Stock Bilirubin Standard, 40 mg/100 ml
: Dissolve 0.040 g bilirubin in chloroform at 25°C in a 100 ml volumetric flask and dilute to mark with chloroform at 25°C. Transfer to 30 ml brown glass bottles and store in refrigerator.

Working Bilirubin Standard, 4 mg/100 ml
: Dilute 20μl stock standard at 25°C with 180μl chloroform at 25°C. Prepare fresh just before use.

Diazo Tablets
: Diazo tablets can be obtained from any chemical supply company.

Diazo Reagent
: Place diazo tablet in 50 ml volumetric flask. Add 1.5 ml half-concentrated hydrochloric acid. Complete volume to mark. Prepare fresh daily.

Hydrochloric Acid Reagent Blank
: Dilute 1.5 ml concentrated HCl to 100 ml with distilled water.

Purified Methanol
: Store in 30 ml glass bottles.

REFERENCES

Malloy, H. T., and Evelyn, K. A.: J. Biol. Chem., 119, 481, 1937

van den Bergh, A. A. H., and Grotepass, W.: Brit. Med. J., 1, 1157, 1934

Technical Bulletin No. 6078C

Method of Analysis with the Beckman Model 150 Ultramicro Analytical System

URIC ACID

Ultramicro Adaptation of the Method of Caraway

PRINCIPLE

Two stable reagents, sodium carbonate and dilute phosphotungstic acid, are added to a protein-free tungstic acid filtrate of serum to react quantitatively with uric acid to produce a blue color, the absorbance of which is measured by means of a spectrocolorimeter at 650 mμ.

PROCEDURE

1. Using 400μl. micro test tubes, prepare blank, working standard and unknown.

	Blank	Standard	Unknown
Distilled Water	20μl.*		
Standard		20μl.	
Serum			20μl.
3.2% Sodium Tungstate	80μl.	80μl.	80μl.
0.2N Sulfuric Acid	80μl.	80μl.	80μl.

*The same pipette is used for blank, standard and serum. Rinsing is done by allowing one drop of sample to overflow from the pipette before an aliquot is removed.

2. Mix thoroughly and centrifuge about 15 seconds. Mix, either by flicking wrist, then tapping bottom of tube; or, alternatively, use Micromixer. Using the transfer pipette, withdraw 100μl. of the clear supernatant and transfer to clean micro test tubes.

	Blank	Standard	Unknown
Transfer pipette	100μl.	100μl.	100μl.
Sodium Carbonate	20μl.	20μl.	20μl.
	Mix	Mix	Mix
Dilute Phosphotungstic Acid	20μl.	20μl.	20μl.
	Mix	Mix	Mix

3. Let stand for 30 minutes.
4. Read absorbance of the unknown and standard at wavelength of 650 mμ. on the Beckman/Spinco Model 151 Spectro-Colorimeter against the blank set at zero absorbance.

CALCULATIONS

Multiply the ratio between the Spectro-Colorimeter absorbance reading obtained with the unknown to the reading obtained with standard, by four, to obtain a result expressed in milligrams of uric acid per 100 milliliters of serum. Use this equation:

$$\frac{\text{Unknown}}{\text{Standard}} \times 4 = mg.\ uric\ acid/100\ ml.\ serum$$

REAGENTS

Either prepare reagents as described below, or alternatively, follow the Uric Acid Chempak instructions.

User's Own Chemicals

Stock Uric Acid Standard, 40 mg./100 ml.:
Transfer 0.040 gm. pure uric acid to a 100 ml. volumetric flask. Dissolve separately, 0.46 gm. anhydrous disodium phosphate, Na_2HPO_4, in 60 ml. warm distilled water. Transfer this solution to the volumetric flask and mix until the uric acid dissolves. Cool, add 0.18 ml. glacial acetic acid and dilute to the mark with distilled water. Store in brown 30 ml. polyethylene bottles.

Working Uric Acid Standard, 4 mg./100 ml.:
Dilute 20μl. stock standard with 180μl. distilled water in a 400μl. micro test tube. Mix thoroughly. Prepare fresh daily.

Sodium Carbonate, 10%:
Dissolve 10 gm. Na_2CO_3 in distilled water and dilute to 100 ml. Store in 30 ml. polyethylene bottles.

Sodium Tungstate, 3.2%:
Dissolve 3.2 gm. $Na_2WO_4 \cdot 2H_2O$ in distilled water and dilute to 100 ml. Store in 30 ml. polyethylene bottles.

Sulfuric Acid, 0.2N:

Add 0.55 ml. of concentrated H_2SO_4 to distilled water and dilute to 100 ml. Store in 30 ml. polyethylene bottles.

Phosphotungstic Acid:

Dissolve 10 gm. molybdate-free sodium tungstate, $Na_2WO_4 \cdot 2H_2O$, in 80 ml. distilled water in a florence flask. Add 8 ml. 85% phosphoric acid and reflux gently for 2 hours. Cool and dilute to 100 ml. Store in brown 30 ml. polyethylene bottles.

Dilute Phosphotungstic Acid:

Dilute 10 ml. of above to 100 ml. with distilled water. Store in brown 30 ml. polyethylene bottles.

Uric Acid Chempak: How to Mix Chemicals

Working Standard:

1. Using the STOCK TRANSFER pipette, transfer 20μl. of STOCK STANDARD to a 400μl. micro test tube.
2. Add 180μl. of distilled water to this tube from the DISTILLED WATER pipette. Mix thoroughly: either flick wrist, then tap bottom of tube, or alternatively, use Micromixer.
3. Prepare fresh working standard daily.

REFERENCE

Caraway, W. T.: Am. J. Clin. Pathol. 25, 840, 1955

Technical Bulletin No. 6079E

Method of Analysis with the Beckman Model 150 Ultramicro Analytical System

PHOSPHORUS

Ultramicro Adaptation of the Method of Fiske and Subbarow

PRINCIPLE

The serum proteins are precipitated with trichloroacetic acid. The protein-free filtrate containing phosphates reacts with molybdic acid forming phosphomolybdic acid, which in turn is reduced by aminonaptholsulfonic acid to the lower oxides of molybdenum blue. The color intensity is proportional to the amount of phosphorus, which is present as phospate ion.

PROCEDURE

1. Using 400μl. micro test tubes, prepare blank, working standard and unknown.

	Blank	Standard	Unknown
Distilled Water	20μl.*		
Standard		20μl.	
Serum			20μl.
Trichloroacetic Acid	150μl.	150μl.	150μl.
	Mix	Mix	Mix

*The same pipette is used for blank, standard and serum. Rinsing is done by allowing one drop of sample to overflow from the pipette before an aliquot is removed.
**Mix either by flicking wrist, then tapping bottom of tube; or, alternatively, use Micromixer.

2. Let stand two minutes.
3. Centrifuge 1 minute.
4. Transfer aliquot of supernatant to clean micro test tubes.

	Blank	Standard	Unknown
Transfer Pipette	100μl.	100μl.	100μl.
Molybdic Acid	20μl.	20μl.	20μl.
	Mix	Mix	Mix
Aminonaphtholsulfonic Acid	20μl.	20μl.	20μl.
	Mix	Mix	Mix

5. Let stand for 30 minutes, and then read at once. This time interval is critical. Read absorbance of the unknown and standard at wavelength of 650 mμ. on the Beckman/Spinco Model 151 Spectro-Colorimeter against blank set at zero absorbance.

CALCULATIONS

Multiply the ratio of the Spectro-Colorimeter absorbance reading obtained with the unknown to the reading obtained with standard, by four, to obtain a result expressed in milligrams of elemental inorganic phosphorus per 100 milliliters. Use this equation:

$$\frac{\text{Unknown}}{\text{Standard}} \times 4 = mg.\ P/100\ ml.$$

REAGENTS

Either prepare reagents as described below; or, alternatively, follow the Phosphorus Chempak instructions.

User's Own Chemicals

Stock Phosphorus Standard, 40 mg./100 ml.:
Dissolve 0.175 gm. potassium dihydrogen phosphate, KH_2PO_4 (dried overnight at 80°C and cooled in a desiccator), in 100 ml. of distilled water. Keep under toluene. Store in brown 30 ml. polyethylene bottles.

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Working Phosphorus Standard, 4 mg./100 ml.:
Dilute 20μl. stock standard with 180μl. distilled water in a 400μl. micro test tube. Mix thoroughly. Prepare daily.

Trichloroacetic Acid, 10%:
Dissolve 10 gm. trichloroacetic acid in distilled water and dilute to 100 ml. Store in 30 ml. polyethylene bottles.

Molybdic Acid, 2.5%:
Dilute 4.2 ml. concentrated sulfuric acid with about 50 ml. of distilled water. Dissolve 2.50 gm. ammonium molybdate in the acid and dilute to 100 ml. with distilled water. Store in 30 ml. polyethyene bottle.

ANSA, 0.1%
Dissolve 14.6 gm. sodium bisulfite, $NaHSO_3$, and 0.50 gm. sodium sulfite, Na_2SO_3, in distilled water and dilute to 100 ml. Add 0.100 gm. powdered 1-amino-2-naphthol-4-sulfonic acid and shake until dissolved. Store in brown 30ml polyethylene bottles in a refrigerator. The flaky precipitate which may form when ANSA is refrigerated will go back into solution when the ANSA is warmed to room temperature. If the ANSA is cloudy or discolored, or contains floating particles, filter it with activated charcoal before using.

Phosphorus Chempak: How to Mix Chemicals

Working Standard:

1. Using the STOCK TRANSFER pipette, transfer 20μl. of STOCK STANDARD from below toluene layer to a 400μl. micro test tube.
2. Add 180μl. water to this tube from the DISTILLED WATER pipette. Mix thoroughly.
3. Prepare fresh working standard daily.

Ansa:

1. Pour 25 ml. of warm distilled water into 30 ml. bottle labeled ANSA-SULFITE BLEND.
2. Mix, by shaking bottle by hand, until solution forms.
3. Transfer this solution to brown bottle labeled ANSA.
4. Attach 20μl. pipette to ANSA bottle.
5. Store in a refrigerator. The flaky precipitate which may form when the ANSA is refrigerated will go back into solution when the ANSA is warmed to room temperature. If the ANSA is cloudy or discolored, or contains floating particles, filter it with activated charcoal before using.

REFERENCE

Fiske, C. H., and Subbarow, Y.: J. Biol. Chem. 66, 375, 1925

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Technical Bulletin No. 6080E

Method of Analysis with the Beckman/Spinco Model 150 Ultramicro Analytical System

CREATININE

Ultramicro Adaptation of the Method of Folin and Wu

PRINCIPLE

The procedure given is essentially the Jaffe alkaline picrate reaction. The concentration of creatinine is determined in an aliquot of protein-free filtrate with alkaline picrate. Although this reaction is not specific for creatinine, practically all the chromogenic material in serum appears to be creatinine. Good reproducibility is dependent upon: accuracy of reagent concentration, pH and time and temperature during color development.

PROCEDURE

1. Using 400 μl. micro test tubes, prepare blank, working standard, and unknown.

	Blank	Standard	Unknown
Distilled Water	40μl.*		
Standard		40μl.	
Serum			40μl.
Sodium Tungstate	80μl.	80μl.	80μl.
Sulfuric Acid	80μl.	80μl.	80μl.

*The same pipette is used for blank, standard and serum. Rinsing is done by allowing one drop of sample to overflow from the pipette before an aliquot is removed.

2. Mix thoroughly: flick wrist, then gently tap bottom of each tube; or, alternatively, use Micromixer.
3. Centrifuge all above tubes for 30 seconds.
4. Transfer aliquot of supernatant to clean micro test tubes.

	Blank	Standard	Unknown
Transfer Pipette	100μl.	100μl.	100μl.
Alkaline Picrate	50μl.	50μl.	50μl.

5. Mix: flick wrist, then gently tap bottom of micro test tubes, or, alternatively, use Micromixer. Let tubes stand for 20 minutes in the dark.
6. Read absorbance of the blank, unknown and standard at a wavelength of 520 mμ on the Beckman/Spinco Model 151 Spectro-Colorimeter against *distilled water* set at zero absorbance.

CALCULATIONS

Multiply the ratio of the Spectro-Colorimeter absorbance readings obtained with the unknown minus the blank to the reading obtained with the standard minus the blank, by 5, to obtain a result expressed in milligrams of creatinine per 100 milliliters. Use this equation:

$$\frac{\text{Unknown} - \text{Blank}}{\text{Standard} - \text{Blank}} \times 5 = \text{mg. creatinine/100 ml.}$$

NOTES

1. Up to a concentration of 9 mg% of creatinine, the relationship between concentration and absorbance is linear. For higher values the curve levels off; therefore repeat the test on a 1:1 dilution.
2. The alkaline picrate reagent should be made up immediately before use. It is stable for several hours.
3. The wavelength has been changed to 520 mμ because at 500 mμ the noncreatinine chromogens impair the optical specificity of creatinine.
4. If acetone is used in the cuvette, allow the cuvette to dry for several minutes before introducing the alkaline picrate solution.

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REAGENTS

Either prepare reagents as described below; or, alternatively, follow the Creatinine Chempak instructions. *NOTE: Working standards provided with the Creatinine Chempak are stable and do not have to be kept under toluene.*

User's Own Chemicals

Stock Creatinine Standard, 100 mg./100 ml.:

Dissolve 0.100 gm. creatinine or 0.133 gm. creatinine hydrochloride in 10 ml. of 1N HCl and dilute 100 ml. with distilled water. Store in brown 30 ml. bottles. Keep in refrigerator under toluene.

Working Creatinine Standard, 5 mg./100 ml.:

Dilute 0.5 ml. stock standard to 10 ml. with distilled water. Prepare fresh daily.

Picric Acid:

Re-crystallize reagent picric acid from glacial acetic acid (2 mg. to 3 ml. at 100°C). Filter hot. Filter crystals on a Buchner funnel and let stand 5 days in the open air. Weigh 1.160 gm. re-crystallized picric acid and dissolve in about 9 ml. of water. Adjust to pH 2.0 with 2N sodium hydroxide and allow to stand 24 hours. Re-adjust to pH 2.0 and complete to 100 ml. with water. Store in brown 30 ml. polyethylene bottles.

Alkaline Picrate:

Mix 9 parts picric acid with 1 part 2.5N NaOH. Store in brown 30 ml. polyethylene bottles. The alkaline picrate reagent should be made up immediately before use. It is stable for several hours.

Sodium Tungstate, 3.2%:

Dissolve 3.2 gm. in distilled water and dilute to 100 ml. Store in 30 ml. polyethylene bottles.

Sulfuric Acid, 0.2N:

Add 0.55 ml. of concentrated H_2SO_4 to distilled water and dilute to 100 ml. Store in 30 ml. polyethylene bottles.

Creatinine Chempak: How to Mix Chemicals

Alkaline Picrate:

1. Pipette 4.5 ml. of PICRIC ACID SOLUTION into the ALKALINE PICRATE bottle.
2. Add 0.5 ml. SODIUM HYDROXIDE. Mix thoroughly by shaking by hand.
3. Keep solution in the dark.

REFERENCES

Folin, O., and Wu, H: J. Biol. Chem. 38, 81, 1919

Clark, L. C., and Thompson, H. L.: Anal. Chem. 21, 1218, 1949

5/62 4.5M NP

Technical Bulletin

Pipette Washing

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November 1960

Beckman

Washing Instructions for Sample and Reagent Pipettes Spinco Model 150 Ultramicro Analytical System

CLEANING PRECAUTION

An obstructed Micropipette should not be cleaned with a stylette. Such cleaning will scratch the inner surface and irreparably affect calibration.

REAGENT MICROPIPETTE

1. Particles of dried-out reagent solution sometimes obstruct the pipette. This obstruction may be recognized by the adhesion of droplets of solution to the inner walls of the pipette. When droplets appear, transfer the pipette, leaving it in its dome, to an empty bottle from the Sparepak to prevent contamination of reagent solution in subsequent cleansing. (If the dome and stem are one piece, transfer the entire assembly to an empty bottle.)

2. To unblock the pipette, fill it with a mixture of detergent, such as Alconox, and distilled water. Leave for about one minute.

3. Rinse with plain water, distilled water, and then acetone.

4. If the droplets still do not disappear, fill the pipette with concentrated ammonium hydroxide. Allow it to stand for about 15 minutes, then rinse thoroughly with distilled water. Repeat if necessary.

If blockage prevents the entry of detergent, soak the entire pipette in a bath of ammonium hydroxide or detergent solution. Then continue the cleansing process.

(continued)

SAMPLE MICROPIPETTE

1. Rinse the pipette with distilled water after every 20 deliveries.

2. An invisible film of protein forms on the inside of a pipette used to pipette serum, plasma, and blood. To remove the film, every evening fill the pipette with a fresh solution of 0.5% pepsin in 0.1N HCl. Leave standing overnight. By morning, the digestive action of the pepsin will have removed the unseen film. Expel the cleansing solution and rinse with plain water and then distilled water. Note: 2 g of pepsin and 5 bottles of 0.1N HCl are supplied in the Sparepak.

3. If droplets appear at any time on the inner walls of the pipette, follow the cleansing procedure described for the reagent Micropipette.

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AUGUST, 1964

Published by Spinco Division, Beckman Instruments, Inc., Stanford Industrial Park, Palo Alto, California

Beckman Model 150 Ultramicro Analytical System
MODEL 154B MICROMIXER
Operating Instructions

INTRODUCTION

Thorough mixing of solutions is essential for accurate and reproducible ultramicro analyses. With the Beckman Model 154B Micromixer, the contents of a micro test tube can be rapidly and thoroughly mixed. Versatility of mixing is provided by vortical and vibratory mixing actions of the Model 154B Micromixer. Power requirements for the Micromixer are 115 volts at either 50 or 60 cps.

GENERAL DESCRIPTION

The Agitator

The contents of a micro test tube are mixed by holding the tube in contact with a rapidly-rotating agitator on top of the Micromixer. The synthetic rubber agitator has tapered sides and a concave upper surface. It is mounted on an eccentric shaft driven by the Micromixer motor.

When a tube is held in the concavity of the agitator, a vortex is produced in the tube. This vortical action completely mixes the solution along the entire length of the tube. When a tube is held in the eccentric path of the agitator so the agitator strikes the end of the tube each rotation, a vibratory action mixes the contents of the tube.

On-Off Switch

The On-Off switch controls the power to the Micromixer motor. When the Micromixer is not in use, the instrument should be turned off.

OPERATING PROCEDURE

Because a wide variety of solutions will be mixed, it is impractical to recommend a specific time interval for all mixing. Generally, 10 to 15 seconds mixing time is sufficient.

1. Pipette the solutions to be mixed into the micro test tube and fasten the cap on the tube.

   NOTE: In procedures which call for mixing between pipetting of reagents, the tube need not be capped if vortical mixing is used and the tube is held upright during mixing. This allows one to use both hands for rapid pipetting and reagent mixing functions.

2. Turn on the Micromixer and hold the tube against the agitator in position to achieve the desired mixing action. For optimum mixing action, hold the tube loosely without placing undue pressure on the agitator.

   For vortical mixing, hold the tube (capped or uncapped) in the concavity of the agitator. The vortex level is determined by the vertical position of the fingers on the tube and also is dependent on the flexibility of the tube material. The intensity of the mixing can be controlled by varying the pressure on the tube downward and to the outside of the concavity.

NOTE: An optional method of vortical mixing is achieved by sliding the capped tube back and forth against the tapered side of the agitator. The eccentric rotation of the agitator sets the contents of the tube in motion, gradually producing a uniform vortex.

For vibratory mixing, hold the capped tube in the eccentric path of the agitator so the agitator strikes the end of the tube during each rotation. Since the agitator striking the tube creates a more violent action than the vortical mixing, the vibration method is especially useful for applications requiring the re-suspension of packed precipitates.

3. At the conclusion of the mixings, turn the Micromixer off.

NOTE: The Micromixer also is suitable for mixing the contents of most sizes of glass test tubes.

OPERATING PRECAUTION

For maximum longevity of the Micromixer motor, do not apply excessive pressure on the agitator which can impede its rotation. Continued abnormal stress on the motor will damage the bearings.

MAINTENANCE

Replacement of the Agitator

A spare agitator (Part No. 324031) is provided with the Model 154B Micromixer. If the agitator becomes damaged or abraded, it can be replaced as follows:

1. Slide the worn agitator off the shaft.

2. Force the replacement agitator onto the shaft.

3. Make sure there is sufficient clearance between the agitator base and the surface of the instrument before operating the Micromixer.

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Technical Bulletin UM-TB-008E

Method of Analysis with the Beckman Model 150 Ultramicro Analytical System

BILIRUBIN

Ultramicro Adaptation of the Method of Malloy and Evelyn

PRINCIPLE

Investigations based on van den Bergh and Ehrlich reactions have detected bilirubin in two forms. Bilirubin glucuronide can be measured by a *direct reaction* in an aqueous serum dilution. Alcohol-soluble bilirubin can be determined in a 50% methanolic solution by a *total reaction*, which measures both forms of bilirubin. The addition of diazo reagent to each dilution produces a pink-violet color of azobilirubin proportional to the bilirubin concentration. Spectrophotometric absorbance readings are made at one minute for the direct reaction and at 30 minutes for the total reaction.

APPARATUS

400μl micro test tubes
Bilirubin Micropak
  Sample Pipette: 20μl
  Reagent Pipettes: 30μl, 100μl, 150μl, 250μl
Bilirubin Chempak
Beckman Model 151 Spectro-Colorimeter
Beckman Model 154 Micromixer

REAGENTS: Bilirubin Chempak

Stock Standard

1. Use the point of a knife to pry the lid from the container. Be careful not to lose any of the contents.
2. Drop the container and lid into a dry 125 ml Erlenmeyer flask.
3. Add approximately 70 ml chloroform supplied with the Chempak.
4. Swirl the flask to mix thoroughly. Generally, a solution will be formed within 5 to 10 minutes.
5. Pour solution through a dry funnel into a dry 100 ml glass-stoppered volumetric flask.
6. Wash with several portions of chloroform, adding to the flask. Then dilute to exactly 100 ml at 25°C. Mix by inversion.
7. Dry the brown glass bottles, which contained the chloroform, by inverting for several minutes. Pour the stock standard just prepared, 40 mg/100 ml, into these bottles.
8. Screw the caps on tightly. Store in a refrigerator when not in use.

Working Standard

1. Using the stock transfer pipette, transfer 20μl of stock standard at 25°C to a 400μl micro test tube.
2. Using the chloroform pipette, add 180μl of chloroform at 25°C to the tube. Mix thoroughly with the Micromixer.
3. Prepare fresh working standard just before use. Keep capped and discard the excess.

Diazo Reagent

1. Fill a 50 ml volumetric flask about half full with distilled water. Drop a diazo tablet into the flask.
2. Add 1.5 ml of hydrochloric acid (for diazo).

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3. Dissolve the tablet. Add sufficient water to make 50 ml of solution. Cap the flask and mix by inversion until a solution is formed.

4. Filter the solution and transfer the filtrate into the diazo reagent pipette bottle.

5. Attach 30μl pipette to this bottle.

6. Prepare fresh diazo reagent just before use.

PROCEDURE

1. Prepare fresh working standard and diazo reagent. The working standard is extremely volatile and must be protected from evaporation. It should be prepared immediately before use, capped between aliquots, and discarded after use.

2. Use 400μl micro test tubes for Blank and Standard; Total Blank and Unknown; and Direct Blank and Unknown. The chart at the end of the procedure summarizes the step-by-step directions.

   NOTE: After each pipetting, mix with the Micromixer.

3. Into the Blank Standard tube, pipette:
   250μl of methanol
   30μl of hydrochloric acid
   20μl of standard

4. Into the Standard tube, pipette:
   250μl of methanol
   30μl of diazo reagent
   20μl of standard

5. Into the Total Blank tube, pipette:
   100μl of distilled water
   150μl of methanol
   30μl of hydrochloric acid
   20μl of serum

6. Into the Total Unknown tube, pipette:
   100μl of distilled water
   150μl of methanol
   30μl of diazo reagent
   20μl of serum

   NOTE: Cap the tubes, mix thoroughly, and allow 30 minutes for color development. During this time, proceed with steps 7, 8, 9, and 10.

7. Set the Spectro-Colorimeter at zero absorbance against water. Set the wavelength selector at 540 mμ.

8. Into the Direct Blank tube, pipette:
   250μl of distilled water
   30μl of hydrochloric acid
   20μl of serum

9. *NOTE: Since the direct reading is taken only one minute after the addition of serum, only one direct determination can be done at a time.*

   Into the Direct Unknown tube, pipette:
   250μl of distilled water
   30μl of diazo reagent
   20μl of serum
   CAP TUBE AND MIX IMMEDIATELY.

10. One minute after addition of serum in step 9, read absorbance of Direct and Blank against water.

11. Thirty minutes after preparing Standard, Total, and Blanks, read absorbances against water at 540 mμ. When reading absorbance of alcoholic solutions, use special care to avoid bubbles in the cuvette.

Summarized Procedure

	Standard		Total		Direct	
	Blank	Standard	Blank	Unknown	Blank	Unknown
Distilled Water			100μl	100μl	250μl	250μl
Methanol	250μl	250μl	150μl	150μl		
Hydrochloric Acid	30μl		30μl		30μl	
Diazo Reagent		30μl		30μl		30μl
Standard*	20μl	20μl				
Serum*			20μl	20μl	20μl	**20μl

*The same pipette is used for Blank, Standard, and Unknown. To rinse the pipette, allow one drop of sample to overflow the pipette before removing an aliquot.

**Mix immediately with the Micromixer.

CALCULATIONS

Total Bilirubin

$$\frac{\text{Total Unknown–Blank}}{\text{Standard–Blank}} \times 4 = mg\,total\,bilirubin/100\,ml$$

Direct Bilirubin

$$\frac{\text{Direct Unknown–Blank}}{\text{Standard–Blank}} \times 4 = mg\,direct\,bilirubin/100\,ml$$

NOTES

1. Bilirubin should be determined within two hours after the blood is drawn. Serum should be clear and not hemolyzed.

2. Malloy and Evelyn were first to find that 50% methanol gave the maximum color for the total reaction.

USER'S OWN CHEMICALS

Stock Bilirubin Standard, 40 mg/100 ml
Dissolve 0.040 g bilirubin in chloroform at 25°C in a 100 ml volumetric flask and dilute to mark with chloroform at 25°C. Transfer to 30 ml brown glass bottles and store in refrigerator.

Working Bilirubin Standard, 4 mg/100 ml
Dilute 20μl stock standard at 25°C with 180μl chloroform at 25°C. Prepare fresh just before use.

Diazo Tablets
Diazo tablets can be obtained from any chemical supply company.

Diazo Reagent
Place diazo tablet in 50 ml volumetric flask. Add 1.5 ml half-concentrated hydrochloric acid. Complete volume to mark. Prepare fresh daily.

Hydrochloric Acid Reagent Blank
Dilute 1.5 ml concentrated HCl to 100 ml with distilled water.

Purified Methanol
Store in 30 ml glass bottles.

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van den Bergh, A. A. H., Grotepass, W. *Brit. Med. J. I*, 1157 (1934)

Technical Bulletin UM-TB-007E

Method of Analysis with the Beckman Model 150 Ultramicro Analytical System

CALCIUM

Ultramicro Adaptation of the Method of Diehl and Ellingboe

PRINCIPLE

A dilution of serum in alkali is titrated with EDTA in the presence of calcein. The observed color change from fluorescent yellow-green to a non-fluorescent orange-red indicates that the calcium present has been chelated by the titrant.

PROCEDURE

1. Using the micro titration cups, prepare blank, working standard and unknown.

	Blank	Standard	Unknown
Distilled Water	20μl.*		
Standard		20μl.	
Serum			20μl.
KOH	100μl.	100μl.	100μl.
Indicator	10μl.	10μl.	10μl.

*The same pipette is used for blank, standard and serum. Rinsing is done by allowing one drop of sample to overflow from the pipette before an aliquot is removed.

2. In the above order, mix and titrate with EDTA until the indicator gives an orange-red color. If the blank is already orange-red, it need not be titrated.

CALCULATIONS

Milliequivalents of Calcium Per Liter

Calculate as follows to express results in milliequivalents of calcium per liter (*mEq. Calcium/L.*). Subtract the Microtitrator dial reading obtained with blank from the readings obtained with unknown and standard; then multiply the ratio of net unknown to net standard by 5 to obtain the milliequivalents of calcium per liter. Use this equation:

$$\frac{\text{Unknown}-\text{Blank}}{\text{Standard}-\text{Blank}} \times 5 = mEq.\ Calcium/L.$$

Milligrams of Calcium Per 100 Milliliters

Calculate as follows to express results in milligrams of calcium per 100 milliliters (*mg. Calcium/100 ml.*). Subtract the Microtitrator dial reading obtained with blank from the readings obtained with unknown and standard; then multiply the ratio of net unknown to net standard by 10 to obtain a result expressed in milligrams of calcium per 100 milliliters. Use this equation:

$$\frac{\text{Unknown}-\text{Blank}}{\text{Standard}-\text{Blank}} \times 10 = mg.\ Calcium/100\ ml.$$

NOTES

1. Diehl and Ellingboe were first to use calcein as an indicator. This is a procedure analogous to that employed by Schwarzenbach and others.
2. Calcein may be purchased from G. Frederick Smith Chemical Company, Columbus, Ohio.
3. This is a difficult end-point; accuracy comes with experience. For best results, keep the same geometry between the operator's eye and the titration cup.
4. A very satisfactory procedure for illuminating the calcein endpoint involves the use of a Nicholas illuminator. The lamp is placed so that it projects a concentrated beam of light from a position opposite the operator onto the titration cup, at an angle of about 20° above the horizontal. The cavities in the turntable are half-filled with water before the titration cups are inserted. The water diffuses and reflects the light through the cup, making the fluorescence extremely intense. The endpoint is the disappearance

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of the deep green fluorescence as observed from a position directly over the titration cup.

Two other techniques which are suitable for illuminating the endpoint are:

With a white fluorescent lamp of the goose-neck type. The endpoint is the change in color from yellow-green to orange-red.

With ultraviolet light in the dark. The wavelength should be about 3600 Å. The worker, however, must wear protective goggles or at least regular eyeglasses. The endpoint is the disappearance of yellow-green fluorescence.

REAGENTS

Either prepare reagents as described below; or alternatively, use the Calcium Chempak instructions.

User's Own Chemicals

Working Calcium Standard, 5 mEq./L:
Dissolve 0.025 gm. previously dried reagent grade $CaCO_3$ in 10 ml. of distilled water to which 0.6 ml. of 1.0 N HCl has been added. Dilute to 100 ml. Store in 30 ml. polyethylene bottles.

Potassium Hydroxide, 1.25N:
Dissolve 7.0 gm. reagent grade KOH pellets in distilled water, add 0.050 gm. KCN. Dilute to 100 ml. Store in 30 ml. polyethylene bottles.

Indicator Solution:
Dissolve 0.025 gm. Calcein in 100 ml. 0.25 N NaOH. Store in brown polyethylene bottles, and keep refrigerated when not in use. If indicator turns greenish, replace it.

EDTA, 0.02N:
Dissolve 0.372 gm. disodium dihydrogen ethylenediaminetetraacetic acid in distilled water and dilute to 100 ml. Store in 30 ml. polyethylene bottles.

Calcium Chempak: How to Mix Chemicals

Indicator

1. Carefully remove one of the plugs from a CALCEIN container.
2. Empty the contents into the 30 ml. bottle labeled SODIUM HYDROXIDE.
3. Remove the remaining plug and drop both pieces into the 30 ml. bottle, together with the plug which was removed first.
4. Cap this bottle. Mix by shaking by hand.
5. Pour the colored solution into the 30 ml. bottle labeled CALCEIN.
6. Attach the 10µl. pipette to the CALCEIN bottle.
7. Store indicator in refrigerator when not in use; replace it if it turns greenish.

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ALKALINE AND ACID PHOSPHATASES

Ultramicro Adaptation of the Methods of Bodansky, and Baginski-Zak

This technical bulletin gives a recommended procedure for determining alkaline and acid phosphatases in blood serum with the Beckman Ultramicro Analytical System. Unlike the ten "packaged" determinations, however, a kit of prepared reagents (Chempak) and a kit of pipettes (Micropak) are not available from Beckman for this determination.

Reagents may be ordered from your laboratory supply dealer; instructions for making them up are given below under "Reagents." The required micropipettes with their Beckman part numbers are listed under "Apparatus," and may be ordered from your dealer or directly from the Spinco Division of Beckman Instruments, Inc., Palo Alto, California.

PRINCIPLE

Serum is incubated with beta glycerophosphate substrate and one hour is allowed for enzymatic hydrolysis. The level of serum inorganic phosphate is determined and subtracted from the total phosphate, which includes inorganic serum phosphate in addition to liberated ions due to enzymatic action. The resulting value constitutes the phosphatase activity and is expressed in Bodansky units.

APPARATUS

	Part Numbers
400 μl micro test tubes	314326
Micropipettes: 10 μl (1 required)	315152
20 μl (2 required)	315153
50 μl (1 required)	315156
100 μl (4 required)	315159
180 μl (1 required)	315161
200 μl (1 required)	315162
Beckman Model 151 Spectro-Colorimeter	314100
Beckman Model 152 Microfuge	314300
Beckman Model 153 Microtitrator	315106
Beckman Model 154 Micromixer	315300
Constant Temperature Water Bath	

REAGENTS

Alkaline Substrate

Dissolve 500 mg of sodium beta glycerophosphate and 424 mg of sodium diethylbarbiturate in distilled water in a 100 ml volumetric flask. Dilute to 100 ml with distilled water. This substrate should have a pH of 9.6. Layer toluene or petroleum ether on top of solution and refrigerate for not longer than one month.

Acid Substrate

Dissolve 424 mg of sodium diethylbarbiturate in distilled water in a 100 ml volumetric flask. Add 500 mg of sodium beta glycerophosphate and 5 ml of lN acetic acid (60 ml glacial acetic acid diluted to 1 liter with distilled water). Dilute to 100 ml with distilled water. This substrate should have a pH of 5.0. Layer toluene or petroleum ether on top of solution as a preservative and refrigerate for not longer than one month.

Trichloroacetic Acid, 10%

Trichloroacetic Acid, 5%

Stock Phosphate Standard, 40 mg/100 ml

Dissolve 0.175 gm of potassium dihydrogen phosphate (KH_2PO_4 that has been dried overnight at 80°C and then cooled in a dessicator) in 100 ml of distilled water. Keep under toluene.

Working Phosphate Standard, 4 mg/100 ml

This is a 1:10 dilution of stock. Dilute 20 μl of stock with 180 μl distilled water in a 400 μl micro test tube. Mix thoroughly. Prepare fresh daily.

Ammonium Molybdate Solution, 1%

Dissolve 1 gm of ammonium molybdate tetrahydrate in distilled water in a 100 ml volumetric flask. Dilute to 100 ml with distilled water.

Citrate-Arsenite Reagent

Dissolve 2 gms each of sodium citrate and sodium arsenite in about 80 ml of distilled water in a 100 ml volumetric flask. Add 2 ml of glacial acetic acid, then dilute to 100 ml with distilled water.

Ascorbic Acid Solution, 10%

Dissolve 10 gms of ascorbic acid, Merck USP medium crystals, in distilled water in a 100 ml volumetric flask. Dilute to 100 ml with distilled water. The solution is stable for approximately one week at room temperature.

PROCEDURE

The chart at the end of this section summarizes the step-by-step directions. The same blanks and standard may be used when multiple samples are being analyzed.

1. Use 400 μl micro test tubes for Enzyme Blank, Enzyme, Blank, Standard, and Unknown.
2. Pipette 10 μl of water each into the Enzyme Blank and the Blank tubes.
3. Pipette 10 μl of working standard into the Standard tube.
4. Pipette 100 μl of either alkaline or acid substrate into both the Enzyme Blank and the Enzyme tubes. Equilibrate the Enzyme tube at 37°C.
5. Pipette 10 μl of serum into the Enzyme tube. Mix the Enzyme tube, mix the Enzyme Blank tube, and set timer for 1 hour.
6. Spin the Enzyme Blank and the Enzyme tubes in the Microfuge for 10 seconds, then place them in a 37°C water bath till the end of the hour set in step 5.
7. Pipette 10 μl of serum into the Unknown tube.
8. Pipette 200 μl of 5% trichloroacetic acid per tube into the Blank, Standard, and Unknown tubes. Cap and mix them, allow to stand till later.
9. After the hour, suspend the Enzyme Blank and the Enzyme tubes in ice water.
10. Pipette 100 μl of 10% trichloroacetic acid per tube into the Enzyme Blank and the Enzyme tubes, cap, mix, and let stand for 3 minutes.
11. Centrifuge all tubes for 1 minute.
12. Transfer a 100 μl aliquot from each tube into clean micro test tubes marked Enzyme Blank, Enzyme, Blank, Standard, and Unknown respectively.
13. Add the following reagents to each new tube, one tube at a time. Mix the unstoppered tube after each addition.

    20 μl ascorbic acid, 10%
    50 μl ammonium molybdate, 1%
    100 μl citrate-arsenite

    After all three reagents have been added, cap the tubes and mix completely. Let them stand 15 minutes at room temperature.
14. Set the wavelength selector of the Spectro-Colorimeter to 650 mμ. Read absorbances against water set at 100%T. For values too high to read directly, *either* dilute the serum 1:2 or 1:4 with normal saline solution and redetermine the enzyme activity, correcting for this dilution factor in the calculation, *or* take a 50 μl aliquot of Enzyme Blank and a 50 μl aliquot of Enzyme, add 50 μl of 5% trichloroacetic acid to each tube, then the same quantity of color reagents as before, but multiply the formula by 2, as shown in the equation for diluted specimens under "Calculations."

SUMMARIZED PROCEDURE

	Total Phosphate		Inorganic Phosphate		
	Enzyme Blank	Enzyme	Blank	Standard	Unknown
Water	10 μl		10 μl		
Standard				10 μl	
Substrate alkaline or acid	100 μl	100 μl Equil. at 37°C			
Serum		10 μl			10 μl
	Mix, set timer for 1 hour. Spin, place in 37°C water bath for remainder of hour.				
Trichloroacetic Acid, 5%			200 μl	200 μl	200 μl
			Cap and mix, allow to stand.		
	Suspend tubes in ice water.				
Trichloroacetic Acid, 10%	100 μl	100 μl			
	Cap, mix, allow to stand 3 minutes.				
	Centrifuge all tubes for 1 minute.				
Aliquot	100 μl	100 μl	100 μl	100 μl	100 μl
Ascorbic Acid, 10%	20 μl Mix	20 μl Mix	20 μl Mix	20 μl Mix	20 μl Mix
Ammonium Molybdate, 1%	50 μl Mix	50 μl Mix	50 μl Mix	50 μl Mix	50 μl Mix
Citrate-Arsenite	100 μl Mix	100 μl Mix	100 μl Mix	100 μl Mix	100 μl Mix
	Let stand 15 minutes at room temperature.				
Read absorbances at 650 mμ against water set at 100%T.					

CALCULATIONS

The total phosphate minus the inorganic phosphate constitutes the alkaline or acid phosphatase activity and is expressed in Bodansky units.

For *un*diluted specimens, use the following formulas:

$$\frac{E - EB}{S - B} \times 4 = \text{mg \% total phosphate}$$

$$\frac{U - B}{S - B} \times 4 = \text{mg \% inorganic phosphate}$$

For diluted specimens, use the following:

$$\frac{E - EB}{S - B} \times 4 \times (2 \text{ or } 4 \text{ as diluted}) = \text{mg \% total phosphate}$$

$$\frac{U - B}{S - B} \times 4 = \text{mg \% inorganic phosphate}$$

in which,

U = absorbance of the Unknown
S = absorbance of the Standard
B = absorbance of the Blank
E = absorbance of the Enzyme (Unknown)
EB = absorbance of the Enzyme Blank

NOTES

1. The rate of enzymatic hydrolysis and the optimum pH depend upon the nature of the substrate. The degree of enzymatic inactivation on surfaces is dependent on the nature of the respective apoenzyme. However, there seems to be a negligible increase in both alkaline and acid phosphatase activity in non-wettable micro tubes as compared with glass under the same conditions.
2. Erythrocytes are known to contain large amounts of an acid phosphatase whose greatest activity is at pH 6. Slight hemolysis interferes with the acid phosphatase determination at pH 5.
3. The Bodansky unit of enzyme activity is equal to 1 mg of inorganic phosphate liberated by 100 ml of serum from the respective substrates in one hour at 37°C.
4. Each substrate (acid or alkaline) must have a blank absorbance value, since color development is dependent on changes in salt and acid content. The inorganic phosphorous blank should be colorless.
5. Acid phosphatase is extremely unstable. Store serum in ice or refrigerate prior to analysis.

REFERENCES

Bodansky, A., *J. Biol. Chem.* ***99,*** 197 (1932)

Shinowara, G. Y., Jones, L. M., and Reinhart, H. L., *J. Biol. Chem.* ***142,*** 921 (1942)

Baginski, E. S., and Zak, B., *J. Clin. Chim. Acta* ***5,*** 834 (1960)

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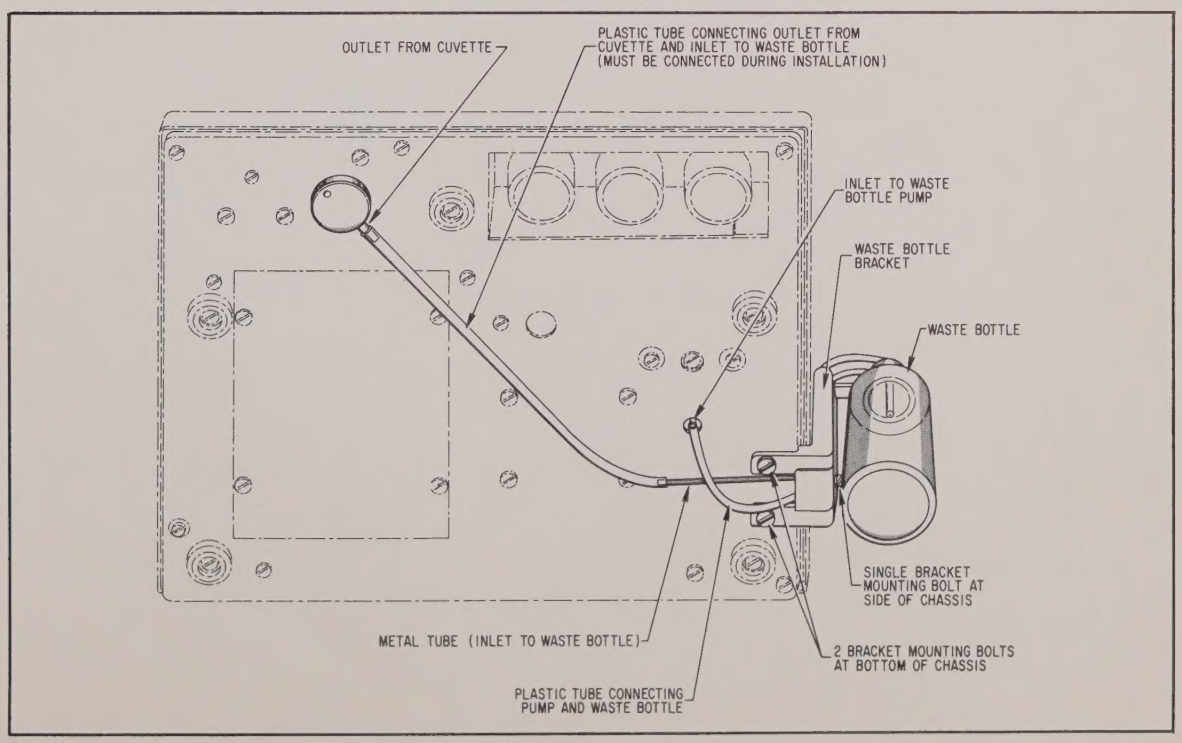
Beckman Model 150 Ultramicro Analytical System

INSTRUCTIONS FOR SETTING UP THE SPECTRO-COLORIMETER

The colorimeter waste bottle is packaged separately from the colorimeter in a cardboard container, along with its bracket, plastic inlet tube, and a small plastic bag containing the two screws which hold the legs of the bracket to the bottom of the colorimeter. The third screw, which holds the bracket to the side of the colorimeter, is already held in place on the bracket by a nut. Remove this nut and discard it. Then set the colorimeter so it is standing on its left end, and set the waste bottle bracket in place as shown in the accompanying drawing. To align the bracket properly, first screw in the two bottom screws tightly, and then tighten the side screw.

Between the legs of the bracket are two tubes, one metal and one plastic. Push the free end of the plastic tube over the inlet to the waste bottle pump, making sure the plastic tube passes *over* the metal tube, as shown in the drawing. If the plastic tube passes under the metal tube, it will be pinched between the metal tube and the bottom of the colorimeter, and the pump will not work properly. Then take the plastic tube which was packaged with the bottle and bracket, and connect one end to the metal tube, and the other end to the outlet from the cuvette. Finally, screw the waste bottle into place on the bracket. *WARNING: Always remove the waste and wash bottles before tipping the colorimeter onto its side or back; otherwise, liquid waste will disable the pump.*



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